



IMPERIAL AGRICULTURAL  
RESEARCH INSTITUTE, NEW DELHI





# ANNALS OF BOTANY

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*ASSISTED BY OTHER BOTANISTS*

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## Miss Ethel Sargent, F.L.S.

THEL SARGANT was born in 1863, and was the third daughter of Henry Sargent, barrister-at-law. She was educated at the North London Collegiate School, under Miss Buss, and at Girton College, Cambridge, where she took Parts I and II of the Natural Science Tripos in 1884 and 1886.<sup>1</sup> She came to Kew in October, 1892, and it was here that her career as a botanical investigator began. From 1893 until the death of her mother in 1912 she lived at home, and for some years was able to give most of her time to research work in her own laboratory. Later, her devotion to an invalid sister and to her mother left her little opportunity for continuous work. In 1912 she took the Old Rectory at Girton, where she spent the last years of her life in surroundings which were especially congenial to her. She died at Sidmouth, after a short illness, on January 16, 1918.

By her premature decease, English botany loses one of its ablest and most devoted students, who had accomplished much, and would doubtless have accomplished still more, if life had been prolonged.

When Miss Sargent came to the Jodrell Laboratory in 1892 her exceptional ability in research work at once showed itself. Her first published paper (1893) was a joint one with the present writer, on the pitchers of *Dischidia Rafflesiana*, chiefly concerned with the physiological anatomy of these organs.

But while at Kew a more characteristic line of work was started—the investigation of the nucleus. For some time, both at Kew and afterwards in the very efficient laboratory which she had built at her home, Miss Sargent engaged with great perseverance in the search for centrosomes; their presence in the higher plants was then one of the burning questions of cytology. Like other observers, she was unable to confirm Guignard's conclusions. Her publications on cytological subjects relate to the formation of the nuclei concerned in reproduction. Her chief results are embodied in two papers in the 'Annals of Botany' on the formation of the sexual nuclei in *Lilium Martagon*—the first on oogenesis, the second on spermatogenesis. She was one of those who contributed to the proof of the striking agreement in these fundamental processes between animals and plants.

Incidentally to her own researches Miss Sargent was able to confirm the important discovery, by the Russian botanist Nawaschin, of double

<sup>1</sup> The writer is indebted to Miss Sargent's brother, Mr. W. L. Sargent, for many particulars of her life.

fertilization in Angiosperms. Preparations showing the twofold nuclear fusions were demonstrated by her to the Royal Society in May, 1899. Her position in this matter may be compared to that of Guignard, whose work, though somewhat earlier in date, was also essentially a confirmation of Nawaschin's results. An interesting résumé of the subject of Double Fertilization was given by Miss Sargent in the 'Annals of Botany' a year later.

Miss Sargent's main lifework falls under two periods, which somewhat overlap. The first, already touched on, was cytological; the second, which occupied the remainder of her scientific career, was anatomical, her subject being the comparative anatomy of seedlings and the conclusions to which it led. The latter period was perhaps the more fertile of the two. This work also was started at Kew, where she began to accumulate her seedling material. The first publication on the subject was a joint paper, with Mrs. D. H. Scott, on the development of *Arum maculatum* from the seed (1898).

Her comparative researches were widely extended and soon led to conclusions of far-reaching importance. A new type of transition from stem to root in *Anemarrhena* (Liliaceae) was described in 1900, and this was followed up, two years later, by a preliminary paper on the origin of the seed-leaf in Monocotyledons, the first statement of her important theory. The following year, 1903, saw the publication of her great mémoire on this subject, one of the most valuable of her contributions which have appeared in the 'Annals'. It is illustrated by seven plates, drawn partly by herself and partly by her friend Miss Agnes Robertson, now Mrs. Arber. While agreeing with Prof. G. Henslow and some other writers in deriving Monocotyledons from Dicotyledons, contrary to the views of the majority of botanists up to that time, Miss Sargent was led to quite an original interpretation of the relations between the two classes. Her comparison of the seedling-structure of certain Liliaceae with that of Dicotyledons, which are exceptional in having a single seed-leaf, indicated that in the former, as well as in the latter, the one cotyledon represents a fused pair. The anatomical facts supporting this conclusion are worked out in the fullest detail. This point then, the origin of the single cotyledon by fusion, is the first essential of the theory. Secondly, while Prof. Henslow had traced the Monocotyledons to an aquatic ancestry, Miss Sargent explained their peculiarities of structure by the hypothesis that they were essentially *geophilous* plants, originally possessing underground stems, such as bulbs, corms, or rhizomes, as so many of them still do.

Thus the Monocotyledons are regarded as having sprung from some early and simple race of Dicotyledons, by adopting, in the first instance, a geophilous habit. Our author was able to offer, on these lines, a satisfactory explanation of all the leading characters of Monocotyledons—the single

seed-leaf, the scattered vascular bundles, the usual absence of secondary growth, and the sheathing leaf-base. In all these points her position is supported by a careful comparison with Dicotyledons of similar habit.

The full memoir appeals, as is natural, to the specialist; for the more general reader there is an admirable summary of the theory in an article on the Evolution of Monocotyledons, published in the 'Botanical Gazette' for 1904. Some years later an elaborate discussion of the whole theoretical position appeared in the 'Annals' (1908) under the title 'The Reconstruction of a Race of Primitive Angiosperms'. Her conclusion is: 'It is probable, therefore, that the Primitive Angiosperms resembled Dicotyledons much more nearly than Monocotyledons in their general features, as well as in stem anatomy and the possession of two cotyledons' (p. 183).

Whether Miss Sargent's theory be ultimately accepted or not, there is no doubt that it has exercised a considerable influence on contemporary botanical opinion. The details are worked out so fully and precisely as to give a solid basis to the hypothesis of the origin of Monocotyledons from a Dicotyledonous stock. At present the weakness of the theory lies in the absence of any palaeontological evidence in its support. The early history of Angiosperms is still unknown, but so far as they have been traced back, Dicotyledons and Monocotyledons appear to be of equal antiquity and to show no signs of convergence. At the same time it would be unfair to lay too much stress on facts which may prove no more than our admitted ignorance of the first stages of Angiospermous evolution. On purely morphological grounds Miss Sargent's theory holds a strong position.

In connexion with her general work on seedlings Miss Sargent paid special attention to the difficult subject of the anatomy and morphology of the Grass embryo; two papers, in conjunction with Mrs. Arber, are devoted to this special investigation (1905 and 1915). An admirable discussion of the position of vegetable embryology was given by Miss Sargent in her able address to the Botanical Section of the British Association in 1913.

We have now run rapidly through Miss Sargent's more important original contributions to science. She gave in addition a few popular addresses, but her serious teaching was limited to a course of lectures at the University of London in 1907 on the Ancestry of Angiosperms; her 'Reconstruction' paper, referred to above, is an abstract of these lectures. Her taste and enthusiasm were all for research, and she was unwilling to allow herself to be distracted from it by teaching. She published two essays of a general character—on 'Women and Original Research' (1900), and on 'The Inheritance of a University' (1901). Two passages from the latter may be quoted: 'The great inheritance, then, of the Universities is the tradition of learning for learning's sake' (p. 7). 'Frenchmen, Scotchmen, Americans have a respect for learning: the Englishman alone asks of what

use it is. The subscription to a new laboratory is wrung from his pockets only by demonstrating that research pays in Germany' (p. 6).

Miss Sargant possessed the gift of style; the clearness and vigour of her exposition were no less characteristic than the accuracy of her observation. This was no doubt due in a large degree to her literary tastes; her brother writes: 'Always a great reader, her retentive memory gave her a wide and accurate knowledge of English Literature.' The writer remembers her saying that a love of books was the first thing necessary for a student—a true view, in his opinion, though one not popular among the modern apostles of science.

Miss Sargant was elected a Fellow of the Linnean Society in December, 1904; she was the first woman to serve on the Council. She was also the first of her sex to preside over a Section of the British Association, and proved herself a most capable and business-like President. At Cambridge she was elected an Honorary Fellow of Girton College in 1913 and also succeeded Mrs. Sidgwick as President of the Federation of University Women; towards the close of her life she devoted much time and labour to compiling the Register of University Women for War Work.

Personally Miss Sargant was a woman of a generous nature, who will be long remembered for her many acts of kindness. Her masculine vigour of intellect was associated with a truly feminine character of the highest type.

D. H. S.

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In completing the above list of Miss Sargent's works the writer has had the kind assistance of Mrs. A. Arber, F.L.S.



## Edward Alexander Newell Arber.

DURING the last quarter of a century Fossil Botany has been pursued in this country with great zeal. Among those who have taken a leading part in its progress is Newell Arber, who in a too short life accomplished a vast amount of work.

Arber was born in 1870, and was the son of Prof. Edward Arber, D.Litt., the well-known authority on English literature. He attended King Edward's High School at Birmingham, where his form-master, Mr. Turner, a good naturalist, first roused his interest in Botany. A long stay at Davos, for the sake of his health, at the age of fifteen inspired him with his lifelong passion for Alpine plants, which culminated in 1910 in his book on Plant Life in Alpine Switzerland. He worked at Mason's College, Birmingham, and at University College, London, and in 1893-4 had a year's gardening at the Chiswick Gardens of the Royal Horticultural Society—a valuable practical experience.

In 1895 Arber went up to Trinity College, Cambridge, and the rest of his life was spent at the University. His object was to devote himself to Botany, but he was already interested in Geology as well, and took both subjects for the second part of the Tripos. It was Prof. McKenny Hughes, always his best friend, who led him to work at fossil plants and appointed him Demonstrator in Palaeobotany in 1899. His earliest publications, however, were on recent Botany, and in part on physiological subjects (1899, 1901). His first palaeobotanical paper (1901) was on Royle's Types from India. Henceforth his main line of work was on the fossils and their evolutionary bearing, though he made an incursion into recent floral morphology in 1903. Much of his research was on Carboniferous Floras and largely of stratigraphical importance; but he was equally at home with impressions and with structural specimens, and made most valuable contributions to the purely botanical side of his subject.

In a paper on the Roots of *Medullosa anglica* (1903), he first demonstrated the structure of the sieve-tubes. In his description of *Cupressino-xylon Hookeri*, 1904, he named and characterized a Tasmanian fossil tree, discovered by Sir Joseph Hooker over sixty years before. In 1905 he described specimens of the seed *Lagenostoma*, showing the external characters and attachment to the rachis. In the same year appeared his British Museum volume on the *Glossopteris* Flora, an important systematic work. A paper on the past History of the Ferns (1906) is of great value,

establishing the group Primofilices for Ferns characteristic of the Primary Rocks.

In 1907 he published, in conjunction with Major Parkin, an important memoir on the Origin of Angiosperms, based on Dr. Wieland's discoveries; this work had the unusual distinction of being translated into German. The same authors wrote on the relation of the Angiosperms to the Gnetales in 1908. In the same year Arber, in conjunction with Hamshaw Thomas, made a valuable contribution to our knowledge of the structure of *Sigillaria*.

A sixpenny booklet on Fossil Plants (1909) is worth mentioning for the beauty of the photographs, well calculated to interest the lay reader in the subject.

Arber was also an authority on Coal, and published a volume on its Natural History in the Cambridge Manuals (1911), which was translated into Russian. Among his later works may be mentioned an interesting article, for a German encyclopaedia, on 'Intermediate Stages between Ferns and Seed Plants' (1913) and a revision of Carboniferous Seed-impressions (1914).

When he died so prematurely on June 14, 1918, he left behind extensive unpublished manuscripts, including a valuable and original essay on the Devonian Floras, now of such urgent interest.

Arber married in 1909 Miss Agnes Robertson, D.Sc., F.L.S., herself a distinguished botanist.

D. H. S.

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# Studies in the Phylogeny of the Filicales.

## VII. The Pteroidae.

BY

PROFESSOR F. O. BOWER, Sc.D., F.R.S.

With forty-three Figures in the Text.

IN the third memoir of this series<sup>1</sup> I suggested that the Leptosporangiate Ferns, exclusive of the Osmundaceae, may be grouped into two phyletically distinct series: the *Superficiales*, in which the origin of the sorus is constantly from the surface of the leaf; and the *Marginales*, in which it is as constantly from the margin. But this distinction, important as it is, cannot be applied with rigid uniformity. Investigation shows that in the course of the evolutionary advance of the *Marginales*, as also in the development of certain individual representatives of that series, the original position of the sori is liable to modification. This was illustrated with clearness in the third memoir, in the case of the Dicksonioid derivatives. *Dicksonia* itself shows a movement of the marginal sorus during its individual development towards the lower surface; but this result becomes much more marked in *Davallia*, and reaches its climax in *Nephrolepis*, or perhaps in *Oleandra*. Here the appearance of the mature sorus is as though inserted intramarginally, that is, on the lower surface of the leaf. The biological reason for the change is readily understood. The protection of the young sorus on the lower surface is more efficient than that of a sorus on the margin.

The fact that such modifications actually do occur may dispose the critical mind altogether to reject the distinction drawn between the two series. But the fact that the receptacle in downward-directed sori such as those of *Dicksonia*, *Odontosoria*, and *Lindsaya* is actually marginal in origin, though deflected downwards as development proceeds, illustrates how the initial steps of the modification may actually be observed in the ontogeny of typical *Marginales*.<sup>2</sup> It is held as probable that a change which can be traced in the ontogeny of certain Ferns may have become stamped permanently upon the descent of others. Accordingly the distinction between the *Superficiales* and the *Marginales* would not be absolute. It would be

<sup>1</sup> Ann. of Bot., vol. xxvii, p. 476.

<sup>2</sup> See Ann. of Bot., vol. xxvii, pp. 457-63.

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based upon the relatively early assumption in descent of the superficial position in the former, and the retention of the marginal position in the latter relatively late, or even to the present day. The Superficiales will then group themselves in relation to the relatively primitive Gleicheniaceae, while the Marginales may be affiliated to the Schizaeaceae; and we may anticipate that certain types will take an intermediate position. The underlying hypothesis would be, that in the ancestry of the Leptosporangiate Ferns the marginal position of the sporangia was prevalent, or perhaps universal.

The Ferns grouped under the comprehensive heading of the 'Pterideae' by Diels<sup>1</sup> present special difficulties in their phyletic treatment from such a point of view as this, because they illustrate more clearly than any other group the gradual departure from the marginal position in the course of descent. I had long recognized this,<sup>2</sup> and have accordingly delayed the detailed study of them till the other main sequences should have been sketched out. This having now been done, the attempt may be made to group these 'Pterideae' phyletically. The best way to approach this will be to start from those types which may be held as relatively primitive. That is the method which has regularly been pursued in these studies. Their relation is ultimately to the Schizaeaceae. This was already suggested by Sir W. Hooker, who remarked<sup>3</sup> that *Mohria* combines the capsules of the sub-order Schizaeaceae with the habit of *Cheilanthes*. On the other hand, Prantl showed<sup>4</sup> that the Schizaeaceae are the typical family of primitive Ferns with a marginal origin of their sporangia. Accordingly certain facts relating to the Schizaeaceae which will help our comparisons will first be given. This will form a necessary introduction to the phyletic study of the Pterideae.

#### SCHIZAEACEAE.

In his monograph on the Schizaeaceae (Leipzig, 1881) Prantl laid a secure foundation for the detailed knowledge of the living representatives of this family, in point of external form, dermal appendages, anatomy, and the position and development of the sporangia. If all these characters be taken into account, together with such additional facts as have been acquired later, the general conclusion follows that *Lygodium* is the most primitive of the living genera; that *Schizaea* occupies a position in certain respects more advanced; and that *Anemia* and *Mohria* are relatively advanced genera. The chief facts have already been summarized elsewhere.<sup>5</sup> Here certain facts relating to anatomical structure and to the sporangia may be added, which may help the comparisons to be made later.

<sup>1</sup> *Natürl. Pflanzenfam.*, i. 4, p. 254.

<sup>2</sup> Compare also Prantl, *Die Farngattungen Cryptogramme u. Pellaea*. *Engler's Jahrb.*, iii, p. 403.

<sup>3</sup> *Syn. Fil.*, p. 436.

<sup>4</sup> *Schizaeaceae*, pp. 39-46.

<sup>5</sup> *Land Flora*, pp. 549-51.

The protostelic state of the adult stem of *Lygodium* stands alone in the family. It may perhaps be correlated with the contracted vascular supply to the leaf.<sup>1</sup> Other genera illustrate more advanced vascular conditions. It has been shown by Boodle for *Schizaea pusilla* and *dichotoma* that the young plant starts from protostely, but it soon advances to the condition of a medullated monostele.<sup>2</sup> In the former species it is not recorded as advancing farther, and Boodle specially states<sup>3</sup> that the central parenchyma 'gives no indication of being of the nature of phloem'. The same has been seen to be the case with *S. rupestris*, which I collected in Australia (Fig. 1). This medullated protostele is completely shut in by the endodermis, which is unbroken even where a leaf-trace is given off (Fig. 2).

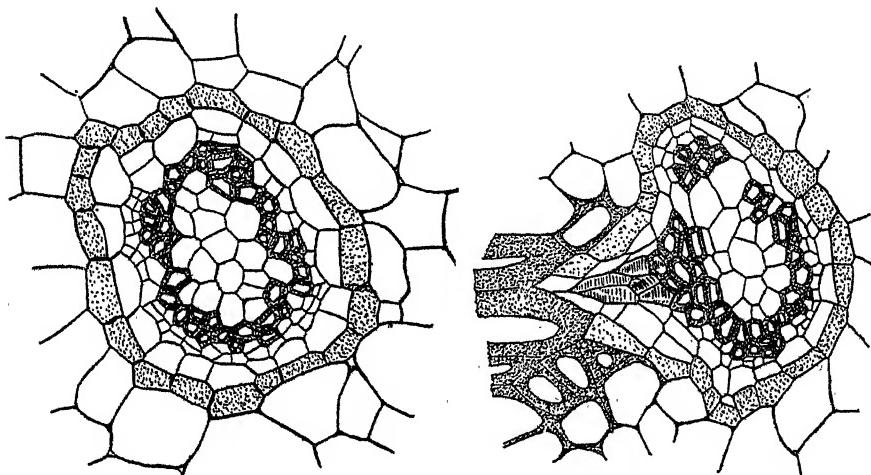


FIG. 1. Transverse section of the stele of an adult axis of *Schizaea rupestris*, showing continuous xylem round a central parenchymatous pith. ( $\times 75$ )

FIG. 2. Ditto, at a point of departure of a leaf-trace upwards, and of a root-trace directed to the left. ( $\times 75$ .)

But while in *S. pusilla* and *rupestris*, and apparently also in *S. fistulosa*,<sup>4</sup> there is, so far as described, no advance beyond the medullated protostele, other species show an internal endodermis, which may intrude downwards as a pouch or pocket from the foliar gaps, but always without internal phloem. This leads to more or less complete 'ectophloic siphonostely', as in *S. dichotoma*,<sup>5</sup> and less perfectly in *S. malaccana*.<sup>6</sup> The imperfection of the 'siphonostely' in the last species has suggested two theoretical explanations.<sup>7</sup> Either that it is reduced, and the imperfect siphonostely is vestigial; or that the structure is an advance on the medullated protostele, and that,

<sup>1</sup> See Gwynne-Vaughan, Ann. of Bot., vol. xxx, 1916, p. 495.

<sup>2</sup> Ann. of Bot., vol. xvii, Fig. 26, p. 525.

<sup>3</sup> Boodle, Ann. of Bot., vol. xv, p. 376.

<sup>4</sup> Tansley and Chick, Ann. of Bot., vol. xvii, Pl. XXV, Figs. 3, 4, 5.

<sup>5</sup> Tansley and Chick, l. c., p. 500.

<sup>6</sup> 1. c., p. 524.

<sup>7</sup> Boodle, l. c., p. 535.

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appearing at the dilated nodes as it does, there is by this means a readjustment of the balance of the intrastelar tissues. If the latter view be accepted, then (i) *S. pusilla*, *rupestris*, and *fistulosa*, (ii) *S. malaccana*, and (iii) *S. dichotoma* would illustrate steps in structural advance of the adult from the protostele actually seen in the juvenile stem to an imperfect solenostely.

In none of these is any internal phloem present. But in *Anemia* it is seen, with the result that in the creeping species (§*Anemiorrhiza*) a typical solenostele is constituted, as in *A. adiantifolia* and *aurita*. In the upright species, however, the leaf-gaps overlap on the abbreviated axis, and typical dictyostely results. This is seen in *A. phyllitidis*, and also in *Mohria*. The successive steps are summed up in the ontogeny of *A. phyllitidis*, as described by Boodle,<sup>1</sup> and early conditions are shown by Fig. 3, i–iv. These early stages are seen to compare with those of *Schizaea rupestris*. But later, as shown by Boodle,<sup>2</sup> an internal phloem appears, giving what is described by Tansley as the *Lindsaya*-structure. It is quickly followed by internal endodermis giving the state of solenostely.

Thus in the Schizaeaceae, more clearly than in any other family of Ferns, the successive steps between protostely and dictyostely are illustrated both in the ontogeny and in the mature state. Those of the more advanced types which have been investigated show more or less clearly in their ontogeny such successive steps of increasing complexity. It is then possible to interpret the adult structure of the less advanced types as having stood still before the progression was completed. This point of view will have its value in the comparative study of the Pteridaceae.

Prantl demonstrated, by hand preparations from all the genera of Schizaeaceae, that the origin of the sporangia is from the marginal cells of the young pinna. This conclusion has since been confirmed for *Lygodium* by Binford,<sup>3</sup> and for *Anemia* by Stevens.<sup>4</sup> I have found his conclusion upheld by sections of the young leaf of *Mohria*, though the marginal position is soon disguised. The question is, however, still open for revision in the case of *Schizaea*.

In the *Synopsis Filicum* (p. 428) this genus is divided into §*Euschizaea*, with frond terete and capsules biseriate: §§*Lophidium*, with frond flattened and capsules biseriate: and §§§*Actinostachys*, with fertile segments digitate and capsules quadriseriate. Already Prantl had investigated *S. pennula*, Sw., which falls into §§§*Actinostachys*, and he found that the sporangia originate on the margin as a single row on each side.<sup>5</sup> He suggested that their appearance as in four rows when mature is due to subsequent displacement. He found them to be covered by indusial flaps, which arise from the upper leaf-surface immediately behind the actual margin, while the sporangia themselves are thereby deflected towards the lower surface.

<sup>1</sup> l. c., p. 389.

<sup>2</sup> l. c., vol. xv, Pl. XX, Figs. 27–30.

<sup>3</sup> Bot. Gaz., vol. xliv, p. 214.

<sup>4</sup> Ann. of Bot., vol. xxv, p. 1059.

<sup>5</sup> l. c., p. 45, Pl. V.

I have been able to trace the stages of development in two species, of which *S. rupestris* appears to be less specialized than *S. digitata*. The

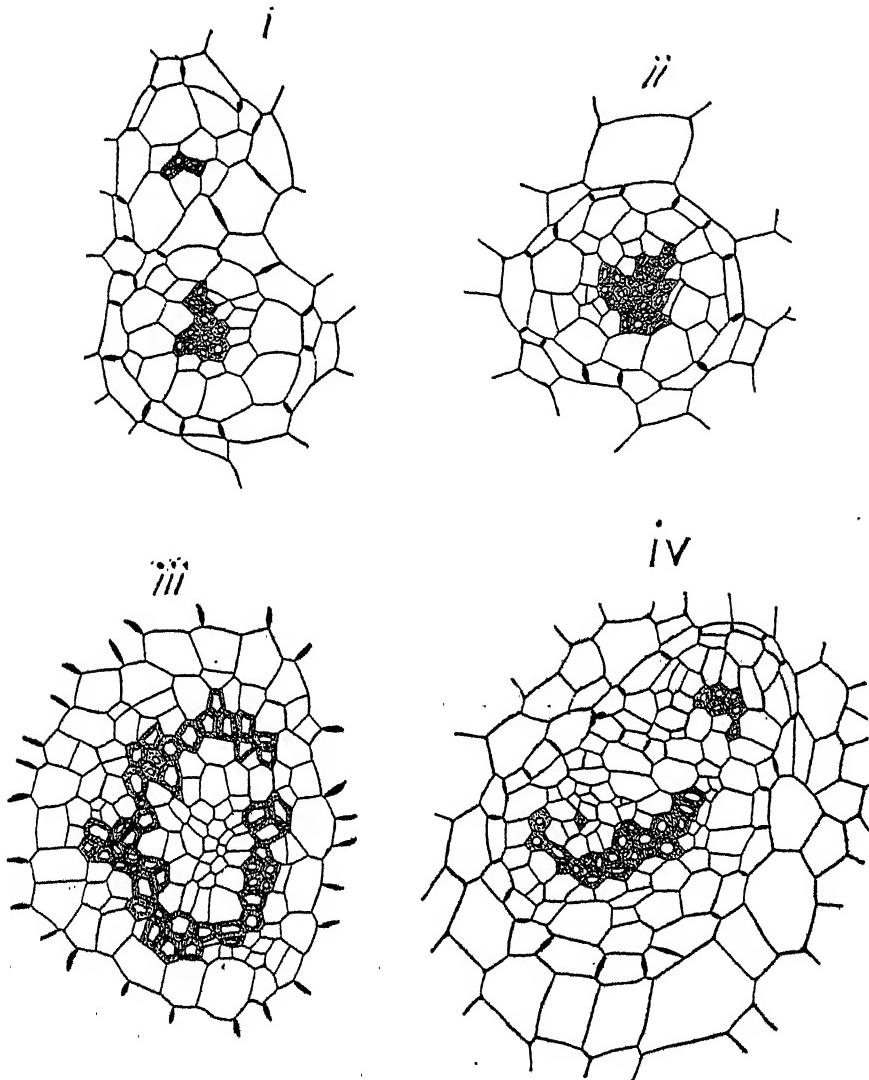


FIG. 3. i-iv. Successive transverse sections from the axis of a young plant of *Anemia phyllitidis*. In i the young protostele has just given off a leaf-trace, but without any interruption of the endodermis; ii shows the protostele rather higher up: iii shows it at a point higher still, with central medulla, as in the sections of *Schizaea rupestris*. In iv the same stele is giving off a leaf-trace, again without interruption of the endodermis. Note the isolated tracheid in the pith. ( $\times 150$ )

material of *S. rupestris* was collected in Australia, chiefly in the district of Wentworth Falls, in the Blue Mountains. It was found growing on rocky ledges, sometimes actually under the spray of waterfalls. The vegetative

leaves are unbranched, like the juvenile leaves of *Marsilia*, and the dichotomies of the fertile leaf are developed on the same scorpioid scheme as the adult sterile leaves of *Marsilia*, differing only in the number of the forkings. These very suggestive parallels strongly support the comparison already

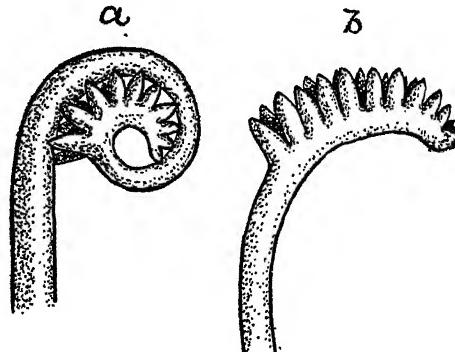


FIG. 4 *a, b.* Young leaves of *Schizaea rupestris*, showing circinate vernation, with the pinnae reflexed to the convex (abaxial) side. ( $\times 6$ .)

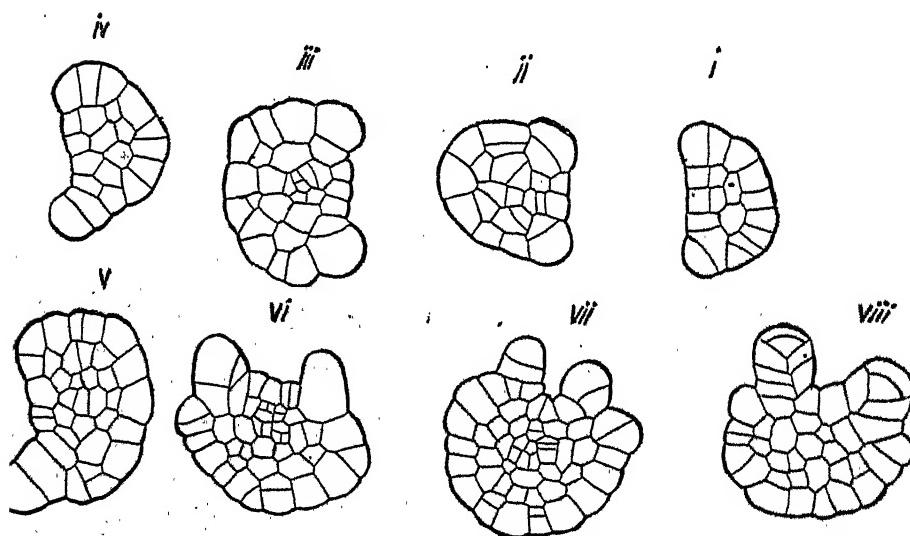


FIG. 5. i-viii. Transverse sections of very young pinnae of *Schizaea rupestris*, showing the marginal origin of the sporangia, which are very soon turned towards the lower (abaxial) surface. In vi, vii the origin of the industrial flaps can be seen, right and left, near the bases of the sporangia. ( $\times 85$ .)

instituted on other grounds. The circination of the young leaves is of the usual type, with the adaxial surface concave; but the pinnae are folded backwards, so that they radiate outwards from the rachis, like the spokes of a wheel. Their lower surface, to which the sporangia are directed, faces inwards (Fig. 4, *a, b*). Transverse sections of the pinnae show the marginal segmentation when very young (Fig. 5, *i*), but almost at once

certain of the marginal cells grow out with increased convexity, and undergo segmentations of sporangial character. A comparison of very young states shown in Fig. 5, i-iv, leaves no room for doubt that the sporangia originate from the margin; while further comparison of older states (v-viii) confirms the sporangial character of the outgrowths. These are, however, very soon diverted sharply to the morphologically lower (ab-axial) surface. Coincidently with this the indusial development begins, and a comparison of the drawings (i-viii) clearly shows that it is secondary. In iii it is indicated by a slight convexity; in vi, vii, viii, segmentations appear in the growth thus formed. In Fig. 6, a, the young indusia are clearly indicated, and in Fig. 6, b, they are already developed to considerable size as protective flaps right and left of the sporangia; their origin having

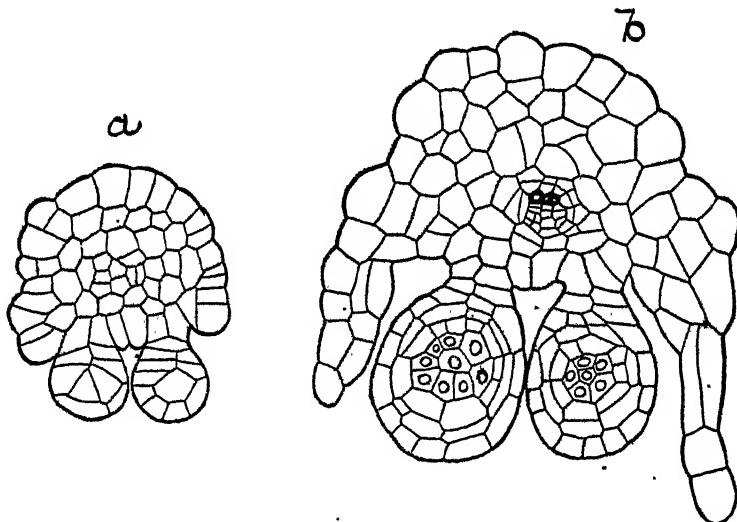


FIG. 6. a, b. Transverse sections of older pinnae, with sporangia and indusia more advanced. ( $\times 85.$ )

been as outgrowths from the upper leaf-surface, just below the marginal sporangia. In this species they attain only a relatively small size, forming an imperfect protection to the sporangia. That the orientation is as described is proved by the position of the xylem, which shows in Fig. 6, b, that the convex side of the pinna is the adaxial. Fig. 7 shows a transverse section of the rachis, with the insertion of two pinnae bearing sporangia. Here also the xylem indicates that the convex side is the adaxial, but it is actually the side of the concave curvature of the rachis as seen in Fig. 4.

A section traversing a single young pinna longitudinally is shown as Fig. 8. The initial cell is seen at the tip, while the section, which was not exactly median, follows one of the lateral rows of sporangia, and these

indicate a slight acropetal sequence. The sporangia themselves follow the characters already well known. If sections be cut transversely to a number of pinnae, their relations to one another appear as in Fig. 9. They alternate in position, and their sporangia dovetail one with another so that collectively a reasonable degree of protection is attained. It may be noted how closely their indusial flaps are placed; and though a careful re-examina-

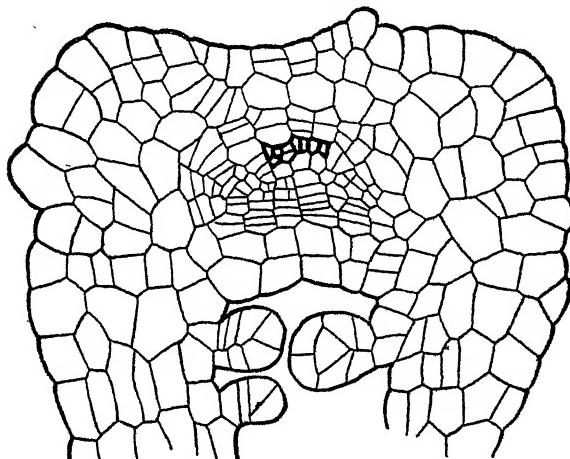


FIG. 7. Transverse section of the rachis and longitudinal of the bases of two pinnae of *Schizaea rupestris*, the xylem indicating the adaxial face. ( $\times 85.$ )

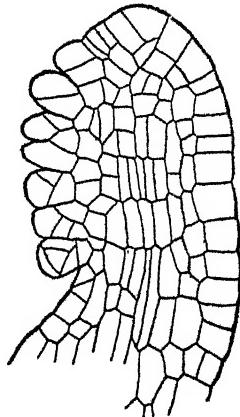


FIG. 8. Longitudinal section of a young pinna of *Schizaea rupestris*, showing the slightly acropetal sequence of sporangia. ( $\times 85.$ )

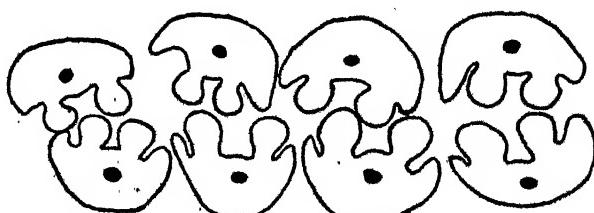


FIG. 9. Transverse section through a number of pinnae, showing their relation to one another. ( $\times 35.$ )

tion of them and of the 'sporocarp' of *Marsilia* will be necessary before any close comparison can safely be made, still it lies close to hand to suggest that by a 'webbing' of the pinnae of *Schizaea* something very like the 'sporocarp' of *Marsilia* would be attained.<sup>1</sup>

The other species examined was *S. digitata*, supplied by the Director of the Botanic Gardens at Singapore. It is interesting as showing in two

<sup>1</sup> There is some variety of detail in the position of the vascular strands in sporocarps of different species of *Marsilia*. These will have to be compared among themselves, and with the vascular arrangement of various species of *Schizaea*, before any conclusion can be reached as to the details of the fusion of lobes suggested, so as to constitute the sporocarp; and the question is further complicated by the receptacular strands present in *Marsilia*. See Duncan Johnson, Ann. of Bot., xii, 1898; Bot. Gaz., xxvi, 1898.

characters an advance on *S. rupestris*. A transverse section of one of the pinnae—which here are fewer in number, but longer than in *S. rupestris*—shows that the indusial flaps are much larger, completely arching in a considerable space on either side, while the sporangia are still relatively small (Fig. 10). The sporangia when mature appear in the four rows characteristic of §§§*Actinostachys*. Prantl has already stated that this is the result of displacement of the sporangia of a single marginal row on each side. Fig. 11, *a*, shows a longitudinal section of the marginal series of cells of a pinna, from which the sporangia arise in acropetal succession. A tangential section of such a marginal series (Fig. 11, *b*) demonstrates

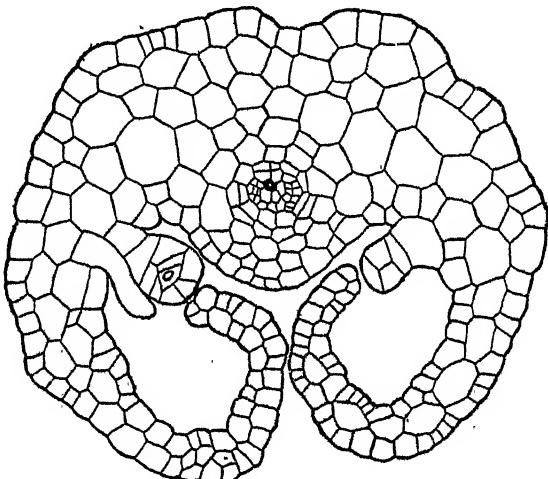


FIG. 10. Transverse section of a pinna of *Schizaea digitata*, showing the indusial flaps much larger in proportion. ( $\times 65.$ )

how by alternate displacements, right and left, the two rows of sporangia are formed from the single marginal series on each side of the leaf. In this, as in the larger development of the indusial flaps, *S. digitata* may be held as an advance upon the type of *S. rupestris*.

This marginal origin of the sporangia of *Schizaea*, and their subsequent displacement and the formation of an intramarginal indusial flap which takes the appearance of a normal leaf-area, are facts thus fully demonstrated for *Schizaea*. The same was also shown by Prantl in *Mohria*.<sup>1</sup> The sporangium arises from the margin; but an intramarginal protective flap originates below it, and finally takes a distal position as though it were a direct continuation of the leaf-surface. The result is that the actually marginal sporangium appears, as it becomes mature, to be superficial. This is shown in Fig. 12, where the vascular strand is seen to run to a point below the sporangial stalk. In some cases it extends beyond it, as noted by

<sup>1</sup> I. c., Pl. VIII, Figs. 134, 135, 138, 140, text, p. 43.

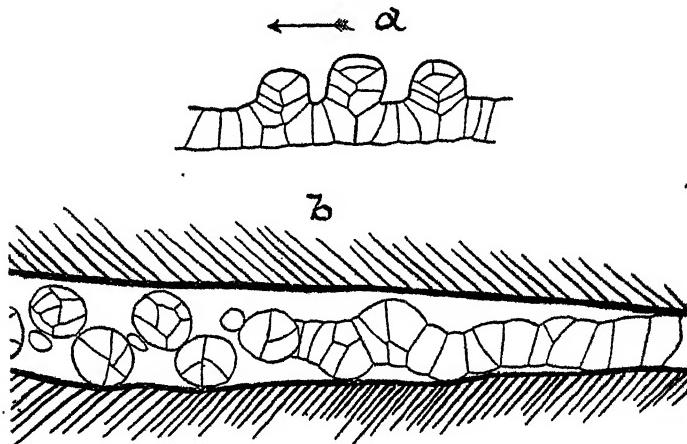


FIG. 11. *a, b.* Longitudinal sections following the marginal series of cells of the pinna of *Schizaea digitata*, and the sporangia which arise there. In *a* they are cut vertically, in *b* horizontally. ( $\times 85.$ )

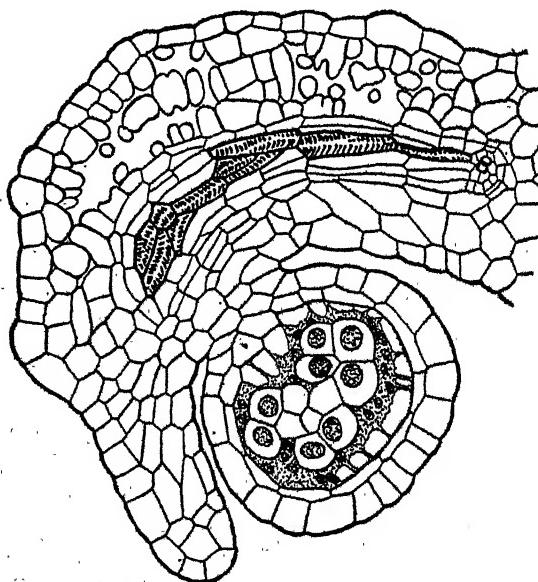


FIG. 12. Longitudinal section through the apex of a pinna of *Mohria caffrorum*, showing the apparently superficial position of the sporangium, which is actually marginal in origin. This appearance is due to the active growth of the indusium from its base, forming a false margin. ( $\times 75.$ )

Prantl;<sup>1</sup> and he delineates it (Fig. 143, C.D.), and remarks that 'this is an indication how one may imagine that a dorsal insertion of the sorus may originate from one terminal on the vein'.

It is thus apparent that, while in all the Schizaeaceae the origin of the sporangia is actually marginal, some of them suggest a transition from the marginal to the superficial position, and *Mohria* provides a pregnant example. Further, it is seen that indusial flaps of various form arise in point of fact superficially, after the marginal initiation of the sporangia which they then protect. Such facts are important for comparison with other types of the Marginales. One related phylum is suggested by *Loxsoma* and the Hymenophyllaceae; another by the Dicksonieae. In both the receptacle and the indusial flaps, which are double, precede the first sporangia in point of time. Their relation to the Schizaeaceae has already been discussed elsewhere.<sup>1</sup> Here it is the Pterideae which will engage special attention, with a view to assigning their approximate phyletic position. It will then be possible to take up more effectively the discussion of the phyletic relations of these and other large series of Ferns, which may be held to have sprung from types in which the sorus was marginal.

#### PTERIDEAE.

The limits and the internal grouping of the Ferns included under this heading have varied in the hands of successive systematists.<sup>2</sup> Though in the main there is naturally some similarity between the several arrangements, it cannot be said that in any one of them the prime object has been a grouping by descent. Systematic convenience and the inclusion under concise and readily handled diagnoses have been more evident in them than the searching out of real affinities. Naturally the systematic grouping must as a rule have the effect of placing related forms in near proximity; but there has been little attempt at the laying out of evolutionary sequences.

Of all the recent Fern-systematists Prantl was the writer who most readily took phyletic views, while his basis for comparison was extended as a consequence of his excellent technique. The result appears in his grouping of the Pterideae, which, though open to amendment, is more acceptable than the rest of his system.<sup>3</sup> He divides his Pterideae thus:

1: *Lonchitidinae*, sorus marginal: spores tetrahedral, or bilateral: hairs simple cell-rows. *Lonchitis*, *Pteridium*, *Paesia*.

2. *Pteridinae*. Sorus superficial, upon the back of the unthickened nerve-endings, and extending thence for various distances downwards. Hairs are flattened scales; spores tetrahedral. *Cheilanthes*, *Pellaea*, *Adiantum*, *Cryptogramme*, *Pteris*.

3. *Gymnogramminae*. Sorus superficial upon the back of the uni-

<sup>1</sup> Land Flora, p. 587.

<sup>2</sup> Compare Presl's Tentamen (1836); Hooker, Synopsis Filicum (1868); Prantl, Das System der Farne (1892); Diels, in Natürl. Pflanzenfam., i. 4 (1902); Christ, Farmkräuter (1897); and Christensen's Index (1906), which follows the grouping of Natürl. Pflanzenfamilien.

<sup>3</sup> Arb. Königl. Bot. Gart. zu Breslau, 1892, pp. 16-17.

thickened nerve, excepting at the apex, and extending for various lengths : hairs either cell-rows or flattened scales : spores tetrahedral. *Pterozonium*, *Jamesonia*, *Anogramme*, *Gymnogramme*, *Nothochlaena*, *Ceratopteris*.

The grading of these three divisions of the Pterideae was primarily on the relation of the sorus to the margin of the leaf. This point had already occupied Prantl's attention among the Schizaeaceae, where he showed that the sporangia are actually marginal. In 1882 he wrote further with regard to certain Pterideae: 'In all the forms quoted, that is in the genera *Pteris*, *Pellaea*, and *Cryptogramme*, as also in *Adiantum* and *Nothochlaena* (*N. Marantae*), the first sporangium appears always at some distance from the leaf-margin ; but in *Cheilanthes* it is quite close to it, so that as the young sporangia develop at first quicker than the margin, the appearance is almost as though (as they actually do in *Mohria*) they sprang directly from the marginal cells.'<sup>1</sup>

Unfortunately this was tested by him only in two species of *Cheilanthes*. When these statements are put in relation with the old remark of Sir W. Hooker<sup>2</sup> that *Mohria* combines the capsules of the order (Schizaeaceae) with the habit of *Cheilanthes*, the suggestion of phyletic relationship directly with that order appears uncommonly strong.

On the other hand, the series with double indusium included in Prantl's Lonchitidinae, which differ widely in habit from *Cheilanthes*, show a soral condition comparable to the doubly-indusiate *Dicksonia-Dennstaedtia* series, in which the marginal origin of the sorus has recently been demonstrated.<sup>3</sup> This suggests a second and probably distinct phyletic line. The first problem before us will therefore be, on the basis of new observations, to see whether there have not been two main lines of descent (possibly more) within the limits of the Pterideae. This will involve the revision of certain developmental and structural characters.

Since we contemplate thus a reasonable probability of two modes of phyletic origin for the Ferns which have been designated Pterideae, it will tend to lucidity of exposition if the Ferns in question are described separately. This is especially desirable because they may appear closely alike in the structure of their sori, though this result may have been arrived at by different channels of evolution. The two series thus contemplated may be provisionally distinguished under the names 'Pterideae bi-indusiatae', or *Pteris series*, in a restricted phyletic sense; and 'Pterideae uni-indusiatae', or *Cheilanthes series*. The former includes those in which there are, or in which there is reason to believe that in descent there have been, two indusia, as in the Dicksonioid-Davalliod-Lindsayoid Ferns ; and the grouping will apply whether or not the one or the other indusium is actually present, for either may be abortive. The latter will include those of

<sup>1</sup> Engler's Bot. Jahrb., 1882, p. 484.

<sup>2</sup> Syn. Fil., p. 436.

<sup>3</sup> Studies, III. Ann. of Bot., vol. xxvii, p. 443.

the Pterideae in which there is only one indusium, and in which there is no reason to believe that there has ever been a second or inner indusium in the course of descent. A central typical genus of the latter is *Cheilanthes*, which gives the proposed name to the series. The present memoir will treat only of the first, which owe their phyletic origin to some source in which the sorus was two-lipped, as in the Dicksonioid Ferns; and the *Cheilanthes* series will be held over for the present.

It is impossible to study the Pterideae satisfactorily within the ring-fence provided by Prantl. The area of comparison upon which their grouping is to be founded must include other Ferns as well, such as the *Dicksonia-Davallia* series. On the basis of a curious over-estimate of the receptacle as a diagnostic character, Prantl separated the Dennstaedtiinae and Davalliinae from one another, and from the Pterideae. It has been shown<sup>1</sup> that the form and construction of the receptacle are related to the arrangement of the sporangia. Basipetal sori have a convex vascular receptacle, and that is seen in *Dennstaedtia* and *Microlepia*; mixed sori have commonly a flat receptacle, and that is seen in *Davallia* and many Pterideae. But such differences are no bar to a relationship which was probably close between the Dennstaedtiinae and Davalliinae and the Pterideae, while all of them converge towards the Dicksonioid type. Such Ferns as these must therefore come into the circle of comparison, and most important of all for our present purposes, *Lindsaya* and *Saccoloma*, on which a few notes may here be given by way of introduction to the Pterideae themselves.

Anatomically the genus *Lindsaya* stands on a relatively primitive footing. Its typical stelar structure is now known for a large number of species, and it may be held as characteristic for the genus, though possibly some exceptions may exist.<sup>2</sup> The *Lindsaya*-type of stele has now been observed in the following species, to which the initials of the observers have been attached: *L. rigida* (A. G. T.), *guianensis* (A. G. T.), *scandens* (A. G. T.: G.-V.), *linearis* (G.-V.: F. O. B.), *stricta* (G.-V.), *orbiculata* (A. G. T.: G.-V.), *cuneata* (G.-V.: F. O. B.), *reniforme* (G.-V.), *Fraseri* (G.-V.), *decomposita* (A. G. T.: G.-V.), *davallioides* (A. G. T.: G.-V.), *ensifolia* (G.-V.), *divergens* (G.-V.), *heterophylla* (G.-V.), *microphylla* (G.-V.: F. O. B.), *lancea* (A. G. T.: F. O. B.), *clavata* (F. O. B.), *cultrata* (F. O. B.: non G.-V.). Its uniformity in these eighteen species suggests that it is a good generic character. There are, however, divergences of observation, depending perhaps on identification of material. Gwynne-Vaughan described for *L. cultrata* a solenostele with divided leaf-trace. But my own material from Singapore shows typical *Lindsaya*-structure for this species. Probably

<sup>1</sup> Phil. Trans., vol. cxii, 1899, pp. 91-5.

<sup>2</sup> Tansley and Lulham, Ann. of Bot., vol. xvi, 1902, p. 157; Gwynne-Vaughan, Ann. of Bot., vol. xvii, 1903, p. 689; Tansley, Lectures on the Filicinean Vascular System, 1908, p. 46.

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Gwynne-Vaughan's material was wrongly identified. There is difficulty also with material identified as *Davallia repens*, of which the anatomy has been described by Trécul, Tansley, and Gwynne-Vaughan as corresponding to that of *Lindsaya*. But I have material sent from the Calcutta Botanic Garden as *Lindsaya repens* which is identified by comparison with herbarium material as *Davallia (Odontoloma) repens*, Desv., in which the vascular structure is exactly that described by Gwynne-Vaughan for his *L. cultrata*. In both cases I think that the *Odontoloma* identification is probably correct. The general conclusion will then be that the primitive *Lindsaya*-type holds for the genus *Lindsaya*, and that *Odontoloma* has solenostelic structure with a divided leaf-trace.

There is, however, a converse anatomical point in this connexion. I collected in Jamaica a Fern named *Davallia clavata* which is ranked in Christensen's Index as *Odontosoria clavata* (L.), J. Sm. It was, however, described as *Lindsaya* by Mettenius.<sup>1</sup> It has a typical *Lindsaya*-stele, and on this account I should uphold its reference by Mettenius as *L. clavata*. The fact is that *Lindsaya*, *Odontoloma*, and *Odontosoria* are very closely related. They include steps of anatomical progression from protostely to solenostely, such as may be illustrated by *L. linearis*, *Odontosoria aculeata*, and *O. retusa*. Any generic line drawn is necessarily artificial.

Finally, the anatomical evidence confirms the exclusion of the so-called *Lindsaya languinosa*, now recognized as *Nephrolepis acutifolia* (Desv.), Christ. For the axis shows two large meristoles, with multiple leaf-traces coming off from foliar gaps right and left, after the fashion of *Davallia*.

It may be noted that dichotomy of the axis has been observed in numerous species of *Lindsaya* and *Odontosoria*.

The general conclusion from these facts is that *Lindsaya* is a genus anatomically primitive, but very closely related to others which are more advanced, a conclusion to which the presence of dermal scales gives support.

But in comparison with the Schizaeoid and Dicksonioid Ferns on the one hand, and the Pterideae on the other, the sorus of *Lindsaya* has a special interest. It has been found in *L. linearis* to be actually marginal in its origin. As in *Dicksonia* the marginal cell by segmentation, as seen in any transverse section, becomes the apex of the receptacle (Fig. 13, i-iii). Two indusial flaps originate intramarginally, as in *Dicksonia*, differing in bulk, the 'inner' (abaxial) being the thinner, while the receptacle itself is slightly deflected to the lower surface. The first sporangia appear upon the extreme margin, and are followed by others showing a slight gradate sequence, especially on the lower side of the receptacle. But this is not strictly maintained, and at an early stage younger sporangia may be interpolated between the older, leading to a mixed condition (Fig. 13, iii) which is quite marked in later stages and involves numerous sporangia.

<sup>1</sup> Fil. Nov. Cal., Ann. Sci. Nat., iv. 15, 1861.

The sori are linked together by vascular commissures which are irregular in their occurrence and extent in different species. This has been shown for *L. lancea* in Studies, III.<sup>1</sup> It thus appears that the sorus corresponds in position to that of *Dicksonia*, but shows an advance in its 'mixed' condition;

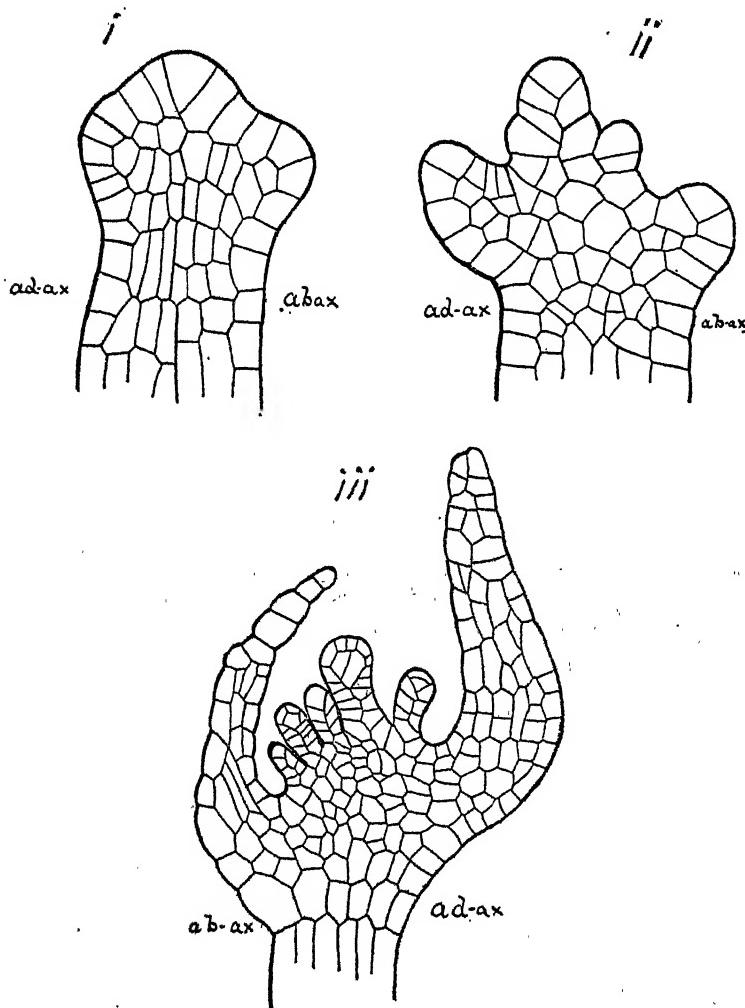


FIG. 13. i-iii. Vertical sections through the young sorus of *Lindsaya linearis*, in successive stages of development : (adax) indicates the adaxial, and (abax) the abaxial side of each. ( $\times 150$ .)

while the inequality of the sides of the receptacle and the introduction of the 'mixed' character, after the initial graduate sequence, are natural advances upon the type of sorus seen in the Dicksonioid Ferns.

Since the sorus becomes a 'mixed' one as it grows older, the oblique

<sup>1</sup> Ann. of Bot., vol. xxvii, p. 460, Figs. 20, 21.

annulus ceases to be so mechanically important as it is in graduate forms like *Dicksonia*. Accordingly the sporangia show varying degrees of obliquity of the annulus. The induration stops opposite the insertion of the stalk, and frequently the series of its cells is interrupted there. But cases may be found where, though the induration stops, the series may still be followed complete past the insertion of the stalk (Fig. 14). These facts correspond with what has been seen for *Lindsaya repens* (Bory), Bedd.<sup>1</sup> In a vertical section of a sporangium it may be seen how an indurated cell of

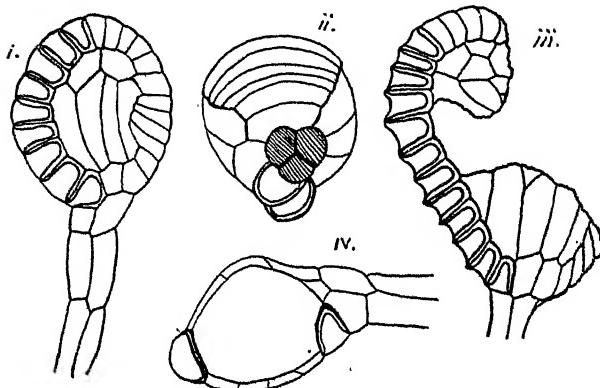


FIG. 14. i.-iv. Sporangia of *Lindsaya linearis* from various points of view. ( $\times 125$ .)

the annulus may actually project into the substance of the stalk itself (Fig. 14, iv). The number of spores in the sporangia of *L. linearis* is 24-32, and the form of the spore is tetrahedral.

#### *Dictyoxiphium.*

It will be best here to introduce that rare monotypic Fern, *Dictyoxiphium panamense*, Hk., first collected by Cumming on the Isthmus of Panama, and figured by Sir W. Hooker,<sup>2</sup> who compared the sorus with that of *Lindsaya*.<sup>3</sup> It is a Fern with habit like a large *Vittaria*, having long lanceolate leaves narrowing at the distal fertile region, where a continuous sorus occupies each margin. The structure of the sorus is not very accurately rendered by Bauer in Hooker's plate quoted above, nor yet by the drawings of sections quoted by Diels from Mettenius.<sup>4</sup> Preparations were therefore made from herbarium material in Glasgow University, from which Fig. 15 has been drawn. This shows that the inner or lower indusium (abaxial) is fully developed and curving upwards envelops the receptacle while young. But the upper or outer indusium (adax), which in *Lindsaya* is the more massive, is here absent. It has been seen that the

<sup>1</sup> Studies, III. Ann. of Bot., vol. xxvii, p. 460, Fig. 22.

<sup>2</sup> Genera Filicum, Tab. LXII.

<sup>3</sup> Syn. Fil., p. 113.

<sup>4</sup> E. and P., i. 4, Fig. 119, B, C.

receptacle in related Ferns is liable to be tilted. In *Lindsaya linearis* (Fig. 13, iii) it is deflected to the lower surface; in *Saccoloma elegans* and *Odontoloma retusa*<sup>1</sup> it is tilted towards the upper. In *Dictyoxiphium* it is still more strongly tilted towards the upper surface, so that, in the absence



FIG. 15. Transverse section of a sporophyll of *Dictyoxiphium panamense*. ( $\times 18$ .)

of the upper indusium, its surface appears almost as a continuation of the upper surface of the leaf. Beneath the receptacle runs a vascular commissure, as in *Lindsaya* or *Pteris*, while it bears numerous sporangia produced in 'mixed' succession. They are long-stalked when mature, and after dehiscence the sporangial head breaks away, leaving the persistent three-rowed stalk.

These facts, stated provisionally until more suitable material can be had, support generally the reference of *Dictyoxiphium* to a relation with *Lindsaya* and with the Pterids. The interest in comparison with the latter lies in the point that while in *Dictyoxiphium* the upper indusium is abortive, in the Pterids (as will be shown below) it is the lower indusium that is reduced, and disappears in the more advanced types. In *Dictyoxiphium* the sorus shows signs of spreading to the upper leaf-surface; in certain of the Pterids it tends to spread to the lower surface; and in *Acrostichum* it may occupy a large part, or even the whole of that surface. Such changes in the originally marginal sorus are similar in kind. They differ in relating the one to the upper, the other to the lower surface of the leaf.

#### *Saccoloma.*

A similar though less complete lateral linking of sori into a marginal series has been described in *Saccoloma elegans*.<sup>2</sup> Here again the origin of the receptacle has been found to be marginal, while there is a one-sided gradation of sporangia upon the receptacle. In fact it is easy to recognize this condition as a modification of the Dicksonioid type, with closely related sporangia shifted to the lower surface. But *Saccoloma* is greatly in advance, anatomically, upon *Lindsaya*; this suggests that their soral states may have resulted from parallel development rather than from phyletic unity. It bears broad brown dermal scales upon its stock.

It is now possible to give some more exact account of the stelar condition of *Saccoloma elegans*, Klf., than has yet appeared. It is based upon material collected in Jamaica, which had been handed over to

<sup>1</sup> Studies, III, Pl. XXXIII, Figs. 16, 17.

<sup>2</sup> Goebel, Flora, Bd. cv (1912), p. 46, Fig. 10. Also Bower, Studies, III, Ann. of Bot., vol. xxvii, p. 457.

Professor Gwynne-Vaughan. As he did not find opportunity to examine it, it was returned to me after his death. From the very hard sclerotic stock sections were cut in series. Fig. 17, *a*–*l*, represent selected sections from one of those series, arranged in succession from below upwards, their actual numbers in the series being marked in plain figures at the base of each drawing. It will be seen that they correspond in essentials to previous descriptions.<sup>1</sup>

The structure of the leaf-trace at the base of the petiole is shown in Fig. 16 as an elaborate horseshoe, accompanied in its sinuous course by outer and inner coverings of sclerenchyma. Right and left are ventilating pneumatodes, while the surfaces of the petiole and stock are covered by broad scales. The continuity of the horseshoe is interrupted on the abaxial side, three strands being quite separate, while the rest of the trace is continuous right and left, extending into deep adaxial hooks. The protoxylem groups are indicated in the drawing by dark dots, and

it will be noted that the three isolated strands contain one each. Thus they represent individual 'divergents'.

The stelar structure of the axis is polycyclic. The outer and middle rings are in this stem complete. The innermost is represented by variable strands. It is probable that in different examples, as also in different species, there may be some latitude of detail. The leaf-trace comes off exclusively from the outermost ring, essentially in the way described by Mettenius. It appears first as

a thinner region of the ring, which soon arches widely outwards, and becomes sinuous in its middle region. Presently the base of the arch becomes narrowed by a sharp angle or kink in the ring on either side, and about the same time the outermost part of the arch is dissociated to form the three strands (divergents) above mentioned, which are separated before the trace is detached from the stele. This differs from Mettenius's account (see his Pl. VI, Fig. 6), as also does the point of detachment. Mettenius represents this as coinciding with the kink or angle already mentioned. But I find that it is constantly below that point (Fig. 17, *a*, *b*, *c*), and that the vascular tissue is continuous at the angles or kinks. Root-steles come off at irregular points from the outer surface of the outer ring, without disturbing it, or having any connexion with the inner system.

The inner ring seems never to be completely closed. It is interrupted

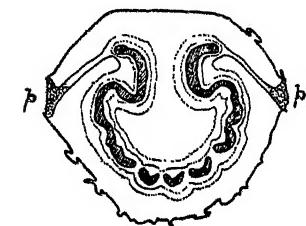


FIG. 16. Transverse section near to the base of the petiole of *Saccoloma elegans*. *p*, *p* = pneumatophores. ( $\times 3$ .)

<sup>1</sup> Karsten, Vegetationsorgane der Palmen, 1847, Pl. IX, Figs. 5 and 6; Mettenius, Bau von *Angiopteris*, Abhandl. K. Sächs. Ges. d. Wiss., Bd. vi, p. 531, Pl. VI, Figs. 1–6. Subsequent writers appear to have used these earlier observations without reinvestigation.

from time to time by giving off 'compensation-strands'. By means of these it is connected to the anterior margin of each foliar gap in the outer ring. The departure of each leaf-trace causes a gap also in the inner ring, which appears usually to be composed of two meristoles of unequal size. The origin of the compensation-strand is seen opposite to the upper foliar gap in Fig. 17, *f*, *g*. Its progress outwards is seen in Fig. 17, *h*, *i*, *j*; and its entry into the foliar gap, which it thus closes, is shown in Fig. 17, *k*, *l*. Other illustrations of the process may be seen in the sections of this series.

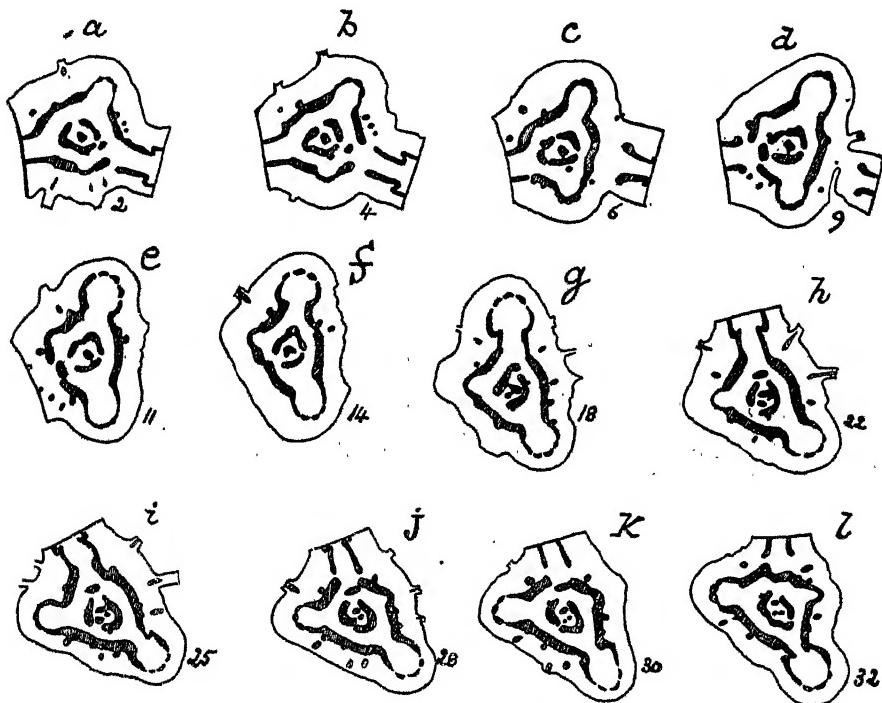


FIG. 17. *a-l.* Series of transverse sections of the stem of *Saccocoma elegans*, in succession from below upwards. The numbers indicate the places of the actual sections drawn in the whole series cut. Natural size.

The attachment of the compensation-strand is comparable to the less complicated case of *Pteris elata* described by Mettenius and depicted by Gwynne-Vaughan.<sup>1</sup> An occasional vascular boss appears on the outer surface of the inner ring (Fig. 17, *b*, *c*, *d*), but it ends blindly, and does not establish connexion with the outer ring. Lastly, the central vascular tract is at times a single, rather thick strand (Fig. 17, *a*, *b*), which establishes connexion with the inner vascular ring (Fig. 17, *c*, *d*, *e*) without disturbing it. But elsewhere considerable irregularities may accompany these fusions, as seen in Fig. 17, *g-l*. It thus appears that the inner system is

<sup>1</sup> I. c., Fig. 14.

less regular than the outer. It will be noted that in the series depicted, two leaf-traces depart, and two fusions occur between the inner and the innermost systems. Thus it appears that one such fusion corresponds to the departure of each leaf.

These facts will be of interest for comparison with those relating to *Pteris podophylla* to be described below.

### PTERIDEAE BI-INDUSIATEAE

#### OR *PTERIS* SERIES IN THE RESTRICTED SENSE.

##### *Pteridium aquilinum* (L.), Kuhn.

Turning now to the Pterideae, it is naturally those with an actually existent double indusium, included by Prantl under Lonchitidinae, which will be taken first. They were held by Prantl himself to be the most primitive.<sup>1</sup> Their soral characters link them clearly with the Dennstaedtiinae and Davallinae, while their simple hairs point to a primitive Dicksonioid relationship. They all show a relatively advanced anatomical condition. There are, however, numerous details which are as yet imperfectly known, even in the familiar Bracken. These require elucidation before the phyletic position can be approached. Meanwhile it may be quoted from Hooker<sup>2</sup> with regard to his §*Paesia*, which includes them, that 'according to strict technical characters this group of species, which differs from the rest of the genus also in habit of growth, has as good a claim to be placed in Lindsayae as Pterideae'. Mettenius also remarked<sup>3</sup> that '*Lindsaya* is distinguished from the indusiate species of *Pteris* by its leaf-margin being unaltered, not rolled back so as to overshadow both sorus and indusium'. It will be noted, however, that these differences are only minor points of adjustment, not valid differences based on initial origin or occurrence of parts. They indicate the near relation of the Lonchitidinae to *Lindsaya*.

The origin of the receptacle of the Bracken has never been fully demonstrated by observation of the actual segmentations. It is true the development has been followed by Burck.<sup>4</sup> The opinions expressed by him, together with his Figs. 26–8, have been quoted as the basis of subsequent statements by Prantl and Diels; and an excellent descriptive abstract is given by Luerssen.<sup>5</sup> But Burck's drawings cannot be held as convincing. Accordingly fresh observations have been made. The material was partly ordinary Scottish Bracken; partly it was of the var. *caudatum*, collected in Jamaica. Their segmentations correspond, and will not be described separately.

<sup>1</sup> I.c., p. 18.

<sup>2</sup> *Syn. Fil.*, p. 162.

<sup>3</sup> *Famgattungen*, iii, p. 6, foot-note.

<sup>4</sup> *Indusium der Varens*, Haarlem, 1874.

<sup>5</sup> *Rab. Krypt.-Flora*, iii, p. 104.

The regular marginal segmentation of the pinna, with a wedge-shaped initial and very strong convexity of the adaxial (upper) face, is shown in Fig. 18, *a-c*. The series of segmentations can be clearly followed, so that the identity of the wedge in *c* is plainly that of the margin in *a* and *b*. In the latter, and more clearly in *c*, the last segment on the adaxial side grows out to form a flap, which is the upper indusium. It is thus clear that it is a superficial, not a marginal growth. Stages slightly older are

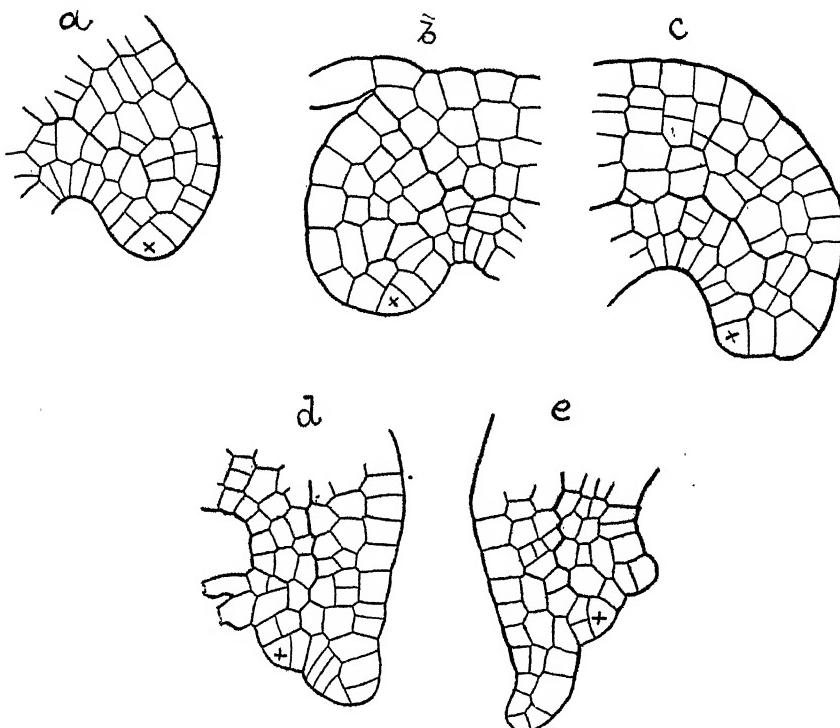


FIG. 18. *a-e.* Sections through the pinnule margin of *Pteridium aquilinum*, showing how the receptacle ( $\times$ ) originates directly from the marginal segmentation, while the indusial flaps are superficial in origin. ( $\times 150$ .)

seen in Fig. 18, *d, e*. The margin here still retains its identity as the cell marked  $\times$ , as shown by the series of segmentations. But now both of the indusial flaps are seen in relation to it. The upper is the stronger, as its earlier origin would indicate. The lower is thinner, less constant in its occurrence and in its structure. It is usually a continuous flap, but sometimes appears as though replaced by a number of distinct hairs. The convexity lying between the two flaps becomes the receptacle, which thus corresponds to that in *Dicksonia*, *Saccoloma*, *Lindsaya*, and *Odontosoria*.

Sections from older pinnules show the sporangia borne on the receptacle. Sometimes there is a regular gradation of these, as is seen in Fig. 19, *a*,

which is comparable with Fig. 13, iii, of *Lindsaya*. But usually the sorus is clearly a 'mixed' one, as in Fig. 19, b, with a rather flattened receptacle, supplied by a mass of storage tracheides. These are connected

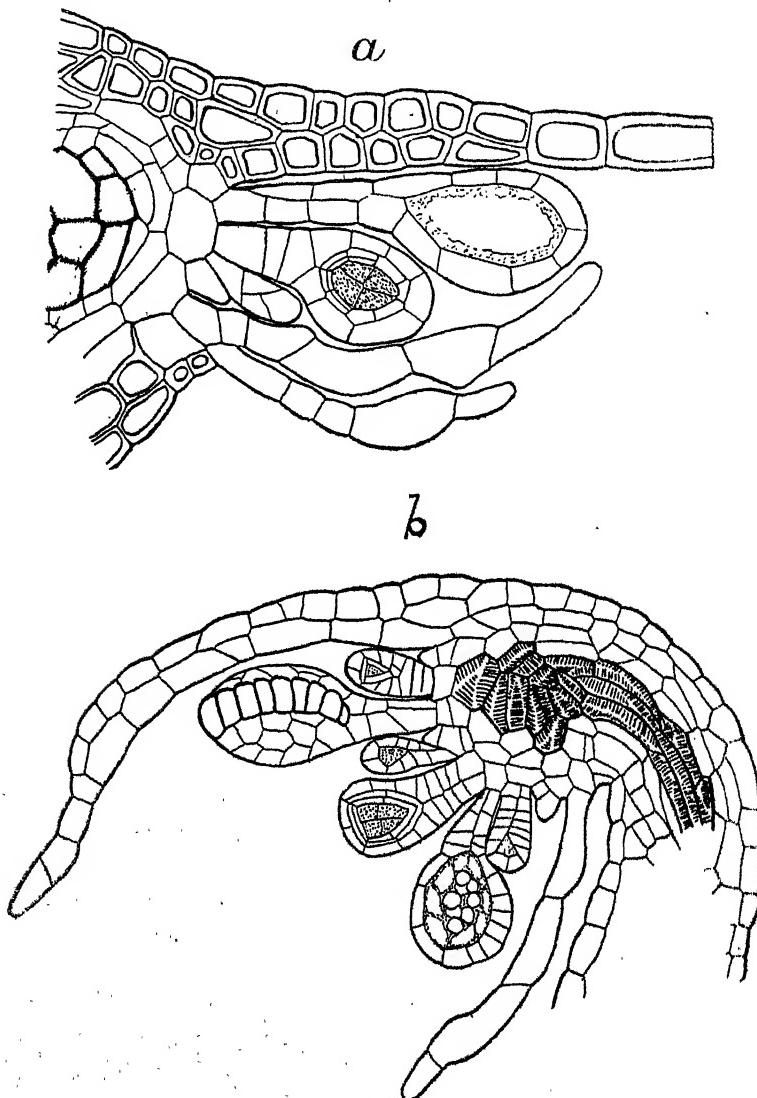


FIG. 19. a. A more mature sorus showing strong basipetal sequence of sporangia in *Pteridium aquilinum*, var. *caudatum*. b. A similar section from Scotch Bracken, showing a mixed sorus. ( $\times 150$ .)

laterally between the veins by the well-known commissures shown in Fig. 20, where also the continuous sequence of the sporangia is shown. From the fully developed soral state as thus described, various steps lead to

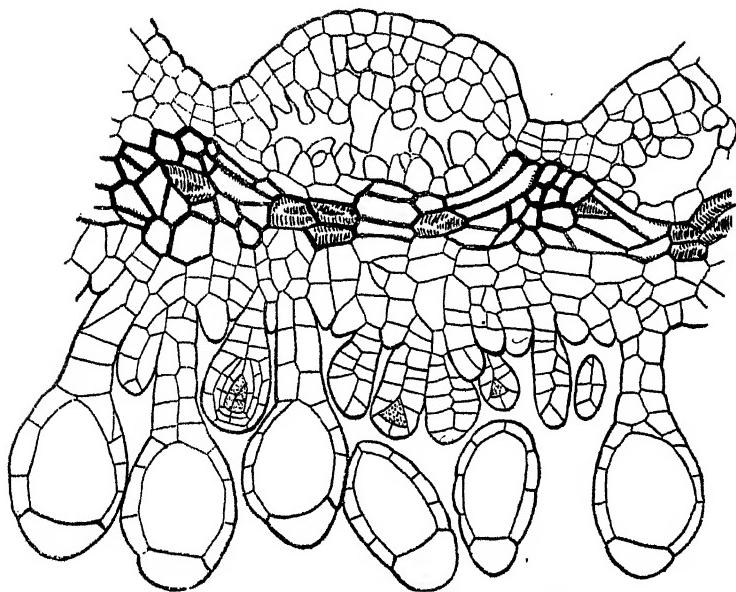


FIG. 20. Vertical section of the fusion-sorus of *Pteriaium aquilinum*, following the line of the commissure. ( $\times 150$ .)

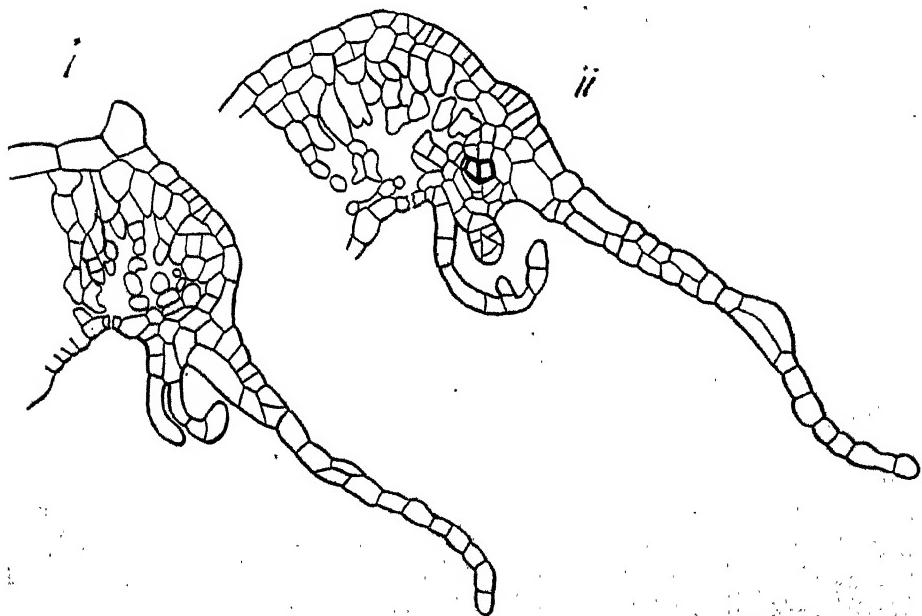


FIG. 21. i, ii. Vertical sections of pinnule of *Pteridium aquilinum*, where the sorus is merging into the sterile margin. ( $\times 75$ .)

the state of the pinnule with sterile margin. Two of these are shown in Fig. 21, i, ii. In the latter both indusia are present and the commissure; but the receptacle is very narrow, and bears only a single sporangium. In i the receptacle, sporangia, and commissure are all absent, though the lower indusium is still represented by hair-like structures. The next step is the absence of the lower indusium also, giving the sterile condition frequently found with upper indusium but without receptacle or commissure, as noted by Luerssen.<sup>1</sup>

### *Paesia.*

*Paesia* has always been associated by systematists with *Pteridium*; in fact the latter has been included in the former genus.<sup>2</sup> The similarity is based partly on habit, but chiefly upon the character of the sorus. The stelar anatomy is simpler than that of *Pteridium*. It is already known for *P. scaberula*. Gwynne-Vaughan has included it in his list of typical solenostelic Ferns.<sup>3</sup> Material of *P. viscosa* was available from Jamaica through the kindness of Mr. Harris. This species bears ample leaves upon its creeping and bifurcating rhizome, and, as in *P. scaberula*, there is a typical solenostele and an undivided leaf-trace. The dermal appendages in both are hairs, as they are also in *Pteridium*, with which the affinity has always been recognized.

The sorus of *P. viscosa* appears tucked away under the margin of the pinnule, and does not usually extend far without interruptions. Several veins may run out to each length of it, and they are connected at their distal ends by a receptacular commissure as in *Pteridium*. The material did not suffice for demonstrating the earliest stages of the sorus, but from comparison of sections of *P. viscosa* with other related Ferns, the receptacle was no doubt of marginal origin. The upper (adaxial) indusium is the larger, and consists of about three layers of cells, thinning out at the margin to a single layer. The lower (abaxial) consists of only a single layer. It arises later than the upper, and is overlapped by it (Fig. 22, B, C). Sometimes it may be altogether absent, as is noted in the specific description in the 'Synopsis Filicum' (p. 163). This is the case in Fig. 22, A, and it may be regarded as an important comparative step towards the state seen in *Pteris*. The first sporangium usually appears on the side of the receptacle nearest to the indusium, and it is followed by a second on the adaxial side. But there is no definite succession, and the number of sporangia is small.

Associated with the sporangia are hairs, but they are not constant. They are multicellular, and the distal cell, though not glandular, is often enlarged and densely protoplasmic (Fig. 22, C). Their appearance in early stages suggests that they may represent abortive sporangia, with

<sup>1</sup> I. c., p. 104.

<sup>2</sup> Syn. Fil., p. 162.

<sup>3</sup> I. c., p. 691.

which interpretation their time and place of origin would accord. The fact of their presence is, however, interesting in connexion with the view expressed by Mettenius, which will be discussed later.<sup>1</sup>

*Paesia scaberula* rarely, if ever, produces fertile leaves in this country. I am indebted to Dr. Cockayne, F.R.S., and other helpers in New Zealand, for material giving the essential features of its sorus. These correspond to what has been described for *P. viscosa*, excepting that the hairs are fre-

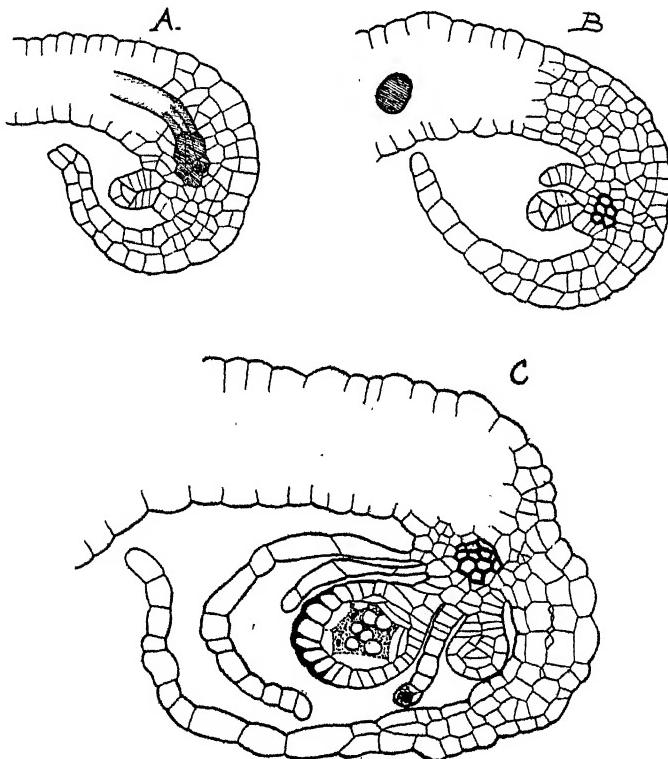


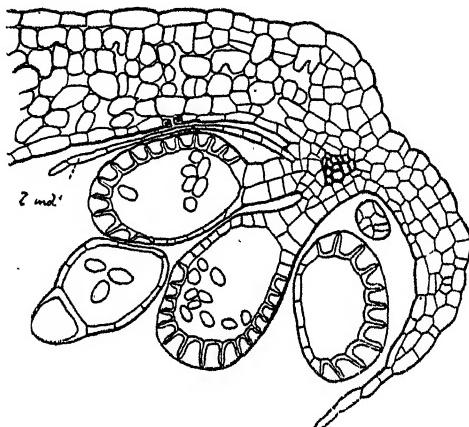
FIG. 22. A-C. Vertical sections of the sorus of *Paesia viscosa*. ( $\times 75$ .)

quently absent. The sorus is either continuous or interrupted, with a marginal commissure. Sections show that it is distinctly inclined to the lower surface. The upper indusium is well developed, but the lower has never been seen thicker than a single layer of cells, and it is closely applied to the lower surface of the leaf (Fig. 22 bis, A). It is also very inconstant in occurrence, and frequently altogether absent. The sporangia originate in basipetal succession, but the series is not long continued. Evidence of this is seen in sections approaching maturity; but

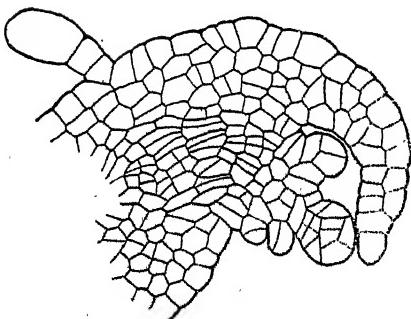
<sup>1</sup> See Mettenius, Farmgattungen, iii, p. 7.

naturally sections of the young state give more direct evidence (Fig. 22 bis, B), the oldest sporangium being seated on the apex of the convex receptacle. Such sections also show the inconstancy of the inner indusium; but it is often difficult to distinguish between the youngest sporangia and the primordium (or vestigium) of the inner indusium. Thus, in Fig. 22 bis, B, it is uncertain whether the lowest abaxial growth is of indusial or of sporangial character. In Fig. 22 bis, C, it is almost certainly an indusium.

A



B



C

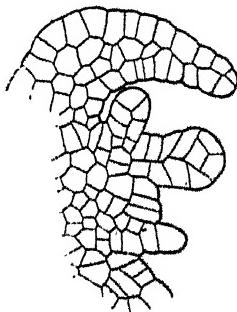


FIG. 22 bis. A-C. Vertical sections through the sorus of *Paesia scaberula*. ( $\times 150$ .)

Thus the general condition of the sorus compares with that of *Pteridium* in being basipetal and typically bi-indusiate; also in the inconstancy of the inner indusium. Material did not suffice for tracing the relation of the receptacle to the marginal segmentation of the leaf. But comparison would indicate with a high degree of certainty that the receptacle is marginal in origin as in *Pteridium*. All the indications point to a very close relation of *Paesia* to that genus.

*Lonchitis.*

The third genus of Prantl's *Lonchitidinae* is *Lonchitis*, of which *L. aurita*, L., has been available from Jamaica. This Fern, which is ranked with *Pteris pubescens*, Willd., by Christ,<sup>1</sup> serves as an example of the strong erect habit, the glandular pubescence of the simple hairs, the reticulate venation, and the position of the sori in the sinuses of the leaf-margin, which are characteristic of the genus. The leaves are arranged spirally on the massive upright stock. The adult stem shows a simple solenostele, from which each leaf-trace comes off as two distinct straps of vascular tissue, separate from one another from the first: an arrangement which finds its parallel in many other Ferns, and is seen in *Pteris cretica*.

The sorus has a rather thick flap of the upper, adaxial indusium, covering the receptacle, on which are sporangia of mixed ages, interspersed with hairs. In the position of the lower, abaxial indusium, which does not exist here as a continuous flap, there appear with some constancy about two ranks of hairs. This suggests a condition where the inner indusium has been resolved into independent cell-rows; though this would not account for the hairs that are interspersed among the sporangia, like those of *Paesia viscosa* (Fig. 22, B), where both indusia are present. These facts are noted in relation to the theory of Mettenius, to be discussed later.<sup>2</sup>

*Lonchitis hirsuta*, L., was described and figured by Sir W. Hooker as *Pteris (Eupteris) laciniata*, Willd.<sup>3</sup> It has a particular interest as illustrating the relation of the indusiate to the non-indusiate Pterids. Hooker states that 'it has the aspect of *Lonchitis*, and is indeed the *Lonchitis hirsuta* of Linnaeus, and of Schkuhr and Willdenow in part'. But 'it exhibits the fructification of a *Pteris*'. 'The affinity of this among the species of *Pteris* is very doubtful.' And in the 'Synopsis Filicum' he remarks (p. 160): 'Though in technical character a *Pteris*, this is far more like the two species of *Lonchitis* in habit.'

Anatomical material collected in Jamaica was handed over to Gwynne-Vaughan, but it was returned to me with some preparations made from it after his death. He had found in the youngest plants a protostele with very parenchymatous xylem, which led upwards to a *Lindsaya*-condition, and later to a solenostele. In the mature stem there is a simple and very large solenostele, over an inch in diameter, with occasional interruptions, apparently of the nature of perforations. In many sections the ring may be complete. The leaf-trace comes off as two distinct straps, separate from one another before detachment from the stele; thus corresponding to that of *L. aurita*, L.

The plant is very hairy, and there are numerous narrow scales on the stock and petiole, though most of the dermal appendages are hairs. The

<sup>1</sup> *Famkräuter*, p. 174.

<sup>2</sup> *Farngattungen*, iii, p. 7.

<sup>3</sup> *Sp. Fil.*, ii, p. 176, Tab. CXXXII.

venation of the leaf is open. The structure of the sorus (shown in surface view in Hooker's Tab. CXXXII) corresponds in essentials of structure, though not in position, to that of *L. aurita*.

The chief point is the absence of an inner indusium, which has been the reason for various authors ranking the plant under *Pteris*.

But it is these intermediate states, leading to synonymy, which provide the foundation for phyletic sequences. The receptacle is very narrow, and the underlying commissure a compact strand. The origin of the sporangia is clearly intramarginal, and their number small, so that their sequence is not obvious. Paraphyses are few or none. It thus appears that in vascular characters, in investiture, in venation, and in soral characters the species of *Lonchitis* occupy an intermediate place between the bi-indusiate types and the genus *Pteris*.

*Pteris (Histiopteris) incisa* (Thunbg.), J. Sm.

This widely-spread species is specially interesting in view of its similarity in habit to *Pteridium aquilinum*, having a creeping rhizome and widely spreading, but glaucous, leaves. Sir W. Hooker<sup>1</sup> notes the variable venation of this Fern. Sometimes it is wholly free, as in *P. aquilinum*; sometimes it is variably anastomosing, after the manner of *Litobrochia*. Though there is a prevalence of hairs, scales do occur on the rhizome. In both of these characters the species indicates an advance on the condition of *Paesia* or *Pteridium*. The habit and dimensions are variable. Sir W. Hooker notes<sup>2</sup> a scandent variety 30 feet long; but the common type is like *Pteridium*, with buried creeping rhizome and upright leaves.

The adult anatomy is already known through the work of Tansley and Lulham.<sup>3</sup> Though less complicated, it shows analogies with the involved structure seen in *Pteridium*. In a younger state it is simply solenostelic, and being a species of considerable size, it seemed a favourable case for studying afresh the ontogeny of a solenostelete. This has already been traced in *P. aquilinum* by Leclerc du Sablon<sup>4</sup> and by Jeffrey.<sup>5</sup> But as neither of these writers has adequately described and delineated the critical point of transition from the *Lindsaya*-condition to solenostely, the detailed observation of it in a favourable example seemed desirable.

Several young plants of *Pteris incisa* collected at Wentworth Falls, Australia, were cut into serial sections, and comparison of them gave the following results. At first, as in *Pteridium aquilinum*,<sup>6</sup> the stele of the axis is seen to have a solid xylem-core, with occasional parenchyma cells associated with the tracheides. As it enlarges upwards, the parenchyma increases at the centre, thus forming a parenchymatous pith. The stele then is composed

<sup>1</sup> Sp. Fil., ii, p. 231.

<sup>2</sup> I. c., p. 231.

<sup>3</sup> New Phyt., iii (1904), p. 1. See also Tansley's Lectures, p. 77.

<sup>4</sup> Ann. Sci. Nat. (1890), Ser. 7, vol. ii, p. 1.

<sup>5</sup> Trans. Can. Inst., vol. vi (1900), Pl. VII.

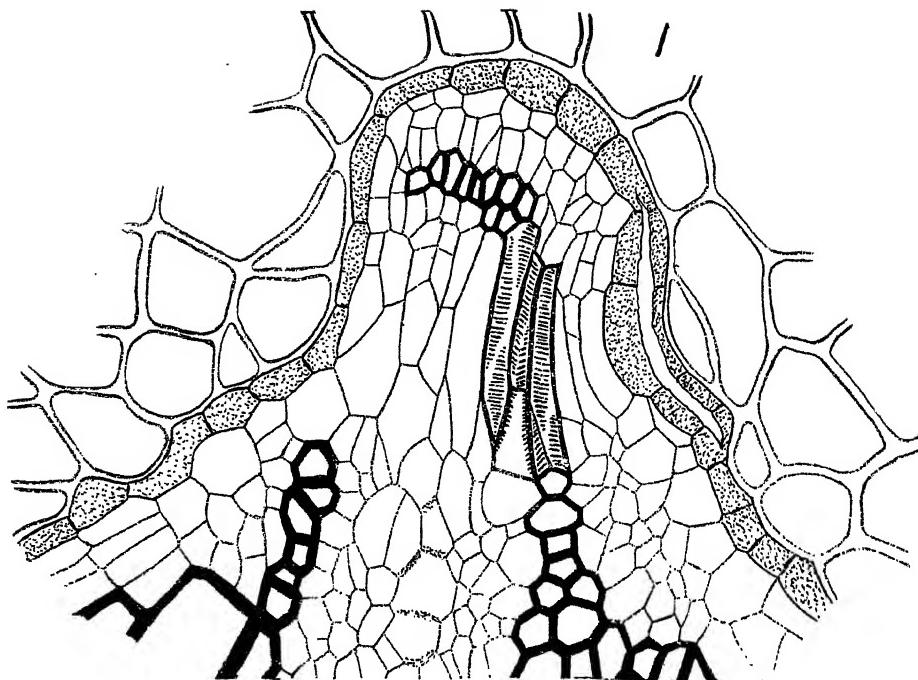
<sup>6</sup> Sablon, I. c., Fig. 3.

of a central pith, a ring of tracheides, usually continuous, but irregular in thickness and in the size of the tracheides.<sup>1</sup> Outside this is a zone of conjunctive parenchyma with phloem, which is at first very scanty: and externally an ill-defined pericycle, and continuous, well-marked endodermis, with cells containing mucilage. When a root-trace departs there is no interruption of the tissues. What happens is, in the case of the medullated stele, that from the outer margin of the xylem-ring—which has previously thickened in preparation—a supply of tracheides comes off, and moves peripherally, and the outer tissues yield. When fully separated from the xylem of the stele, the phloem, pericycle, and endodermis curve inwards behind the outgoing root-supply. Finally the endodermis from either side meets, and the root-supply is thus shut off from the stele without any opening to the external cortex.

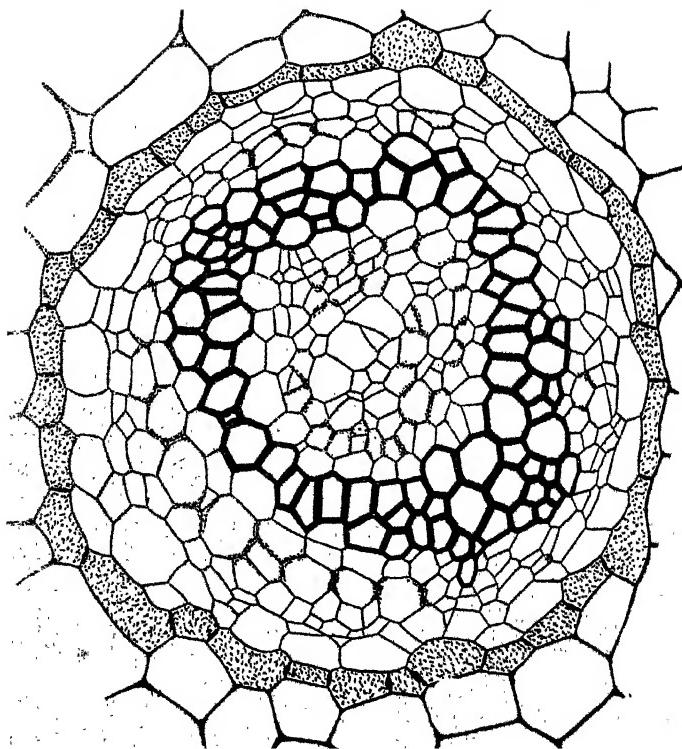
Similarly with the young leaf-trace. Here, however, where the stele is already medullated the xylem-ring opens, and a sector of it with one free margin swings outwards; thus continuity is established between the central tissue and the external conjunctive. The endodermis and pericycle yield as before without any break of continuity, encroaching upon the external cortex. Presently the outward-moving sector is detached from the xylem of the stele and moves obliquely outwards, while the endodermis and pericycle close in behind it, completing the abstraction. Thus in the case both of root and leaf the stele is throughout completely closed. This condition is maintained until a number of leaves have been produced, and the stele has attained considerable size. Meanwhile the quantity of phloem has increased, and it is distributed both inside and outside of the xylem-ring (*Lindsaya*-condition). The two are in continuous connexion whenever a leaf-trace departs.

The transition from this *Lindsaya*-condition to solenostele is the next step. It may be described with illustrations in the case of a definite plant cut as Series E. The departure of the last leaf-trace given off on this relatively simple plan is shown in Fig. 23, I. The endodermis still forms a complete barrier surrounding the obliquely outward-swinging leaf-trace. Certain elements in the central region of the stele are developed as sieve-tubes, which are now present in considerable numbers, in addition to those of the external phloem. The structure of the whole stele of the same plant after this leaf-trace has been abstracted is shown at a higher level in Fig. 23, II, from which it is seen that the stem retains the *Lindsaya*-structure to that point. The next higher leaf-trace which comes off is shown in Fig. 23, III. Here the origin of the leaf-trace and its relation to the stelar tissues are the same as before, but the obliquity is more marked, and the leaf-trace itself larger. With this goes a deeper involution of the endodermis, followed by the thick-walled cells of the

<sup>1</sup> Compare Sablon, *l. c.*, Fig. 4.



II



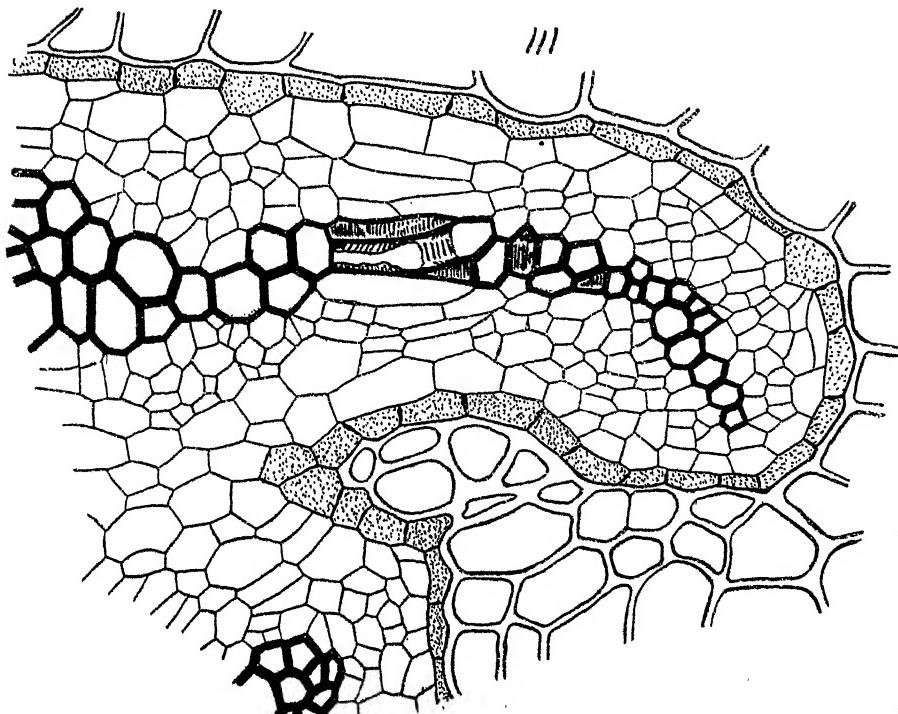


FIG. 23. I-III. Stele and origin of leaf-traces in *Pteris (Histiopteris) incisa*. I shows the departure of the last leaf-trace of the young plant, in which no 'pocket' is formed; II is a section of the stele showing the *Lindsaya*-structure in the internode above that leaf-trace; III shows the involution of the endodermis to form the 'pocket' at the departure of the next higher leaf-trace. ( $\times 150$ .)

cortex. There is in fact the first indication of a 'pocket'. It may be held as a natural consequence of just such an exaggeration of the steps seen in the lower leaves as would result from an increase of size of the leaf and of its trace. The involution gradually smooths out again upwards after the leaf-trace has separated off, thus the pocket is shallow. But before the level of the next higher leaf-trace is reached, sclerotic cells surrounded by an endodermis appear in the centre of the stele, and widening out upwards, communicate at the next leaf-insertion with the outer cortex. This is the first actually complete foliar gap, and from this point onwards such gaps of the usual type occur at each leaf-insertion, the structure being as a consequence solenostelic.

This ontogeny has been described because it illustrates clearly the critical point in that succession of phases of development of the stele which, though variable, is widespread in Ferns. In various cases it may be more or less curtailed, or lengthened. The stages are (1) Protostele, (2) medullated Protostele, (3) *Lindsaya*-condition, (4) Solenostele.

In passing from (1) to (4) in *Pteris incisa* there may be a considerable length of axis, and some half-dozen leaves may be borne. The point which is of the greatest comparative interest is the marked occurrence of a 'Lindsaya-phase'. In the case especially described at least three internodes intervened between the appearance of internal phloem and the establish-

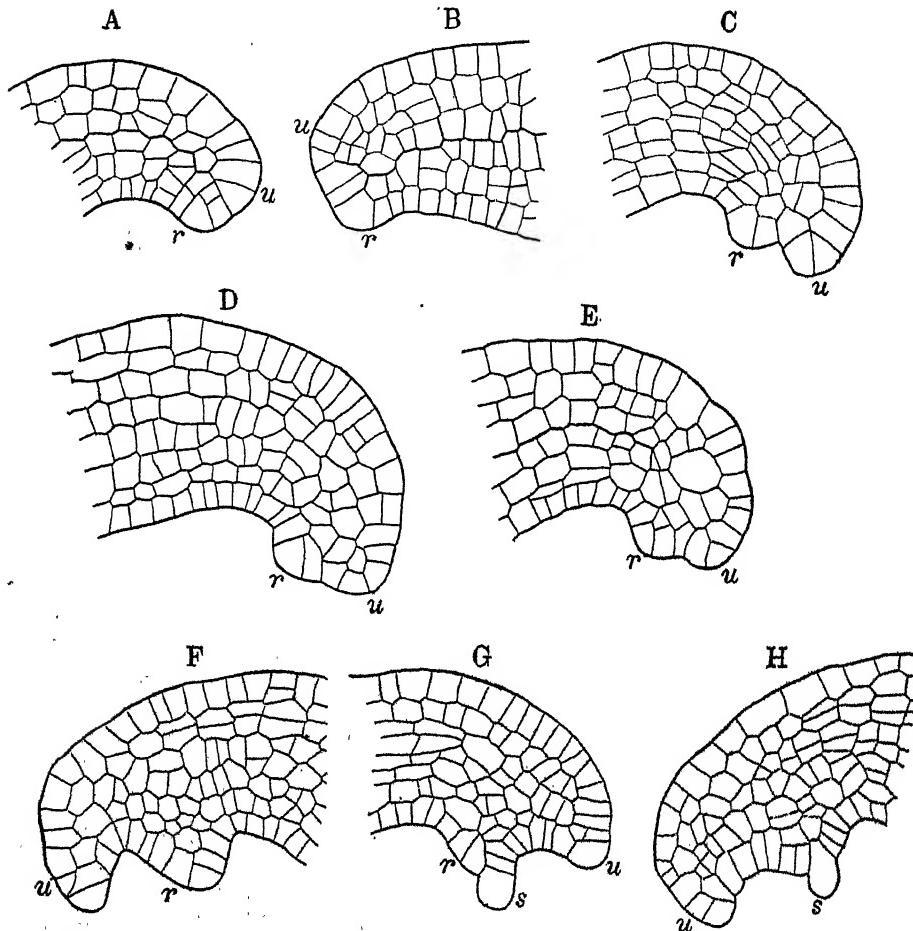


FIG. 24. A-H. Section vertically through the margins of pinnules of *Histiopteris incisa*, showing the relation of soral origin to the marginal segmentation. ( $\times 150$ .)

ment of a continuous solenostelic state. The change is accompanied by a considerable expansion of the stele and enlargement of the leaf-trace. The comparative discussion of these facts will be deferred for the present.

As regards its sorus, *H. incisa* shows advance on *Pteridium* and *Paesia* in the loss of the lower indusium. The habit of the fertile leaf and detail of the sorus have been figured by Mettenius.<sup>1</sup> The origin of the sorus shows

<sup>1</sup> Fil. Hort. Lips., Taf. XIV.

some fluctuation in detail. The segmentation of the sterile pinnule is quite typical. But where a sorus is to be formed the curved outline of the young margin becomes slightly irregular, as seen in the transverse section (Fig. 24, A, B), and the marginal cell appears to be diverted downwards (i.e. to the abaxial surface), owing to stronger growth of the last adaxial segments. Its products soon grow into a projecting ridge, in section conical, which appears then to lie upon the lower surface (Fig. 24, C). The divisions in the last adaxial segments may simulate the marginal segmentation itself, so that it becomes difficult to trace the identity of the real margin. It is possible that in some cases there may have been a slide of the ridge actually to the lower surface. But comparison of a large number of examples leads to the conclusion that the ridge (or in section the cone), which thus appears to be on the lower surface, is essentially, even if not always actually, of marginal origin. It develops as the receptacle ( $r$  in Fig. 24, D, E), while the stronger growth of the adaxial segments has produced the upper indusium ( $n$ , Fig. 24, D, E); the latter is of similar origin to that in *Pteridium* or *Paesia*, or the Dicksonioids generally. As growth proceeds, these parts become more definite in form (Fig. 24, F), and the apparently superficial position of the receptacle more pronounced. From the actual apex of the ridge certain cells project ( $s$ , Fig. 24, G, H). These are the first sporangia, and if the above account of the origin of the receptacle is correct, then these first sporangia are, as in so many of the Marginales, derived from the marginal cells themselves. There is, however, no inner indusium. This was a matter of old observation in the mature state, and was in fact the justification of the inclusion of the species in *Pteris*,  $\S$  *Histiopteris*, now held as a substantive genus. Later sporangia show indications of a basipetal sequence, which, however, is not strictly maintained (Fig. 25, i, ii). With the sporangia hairs (paraphyses) are scattered, and sometimes one may accidentally lie between the receptacle and the lower surface, giving the appearance of a lower indusium (Fig. 25, ii); but this is quite inconstant, and more commonly none is seen there (Fig. 25, i). A vascular commissure runs below the receptacle; it is almost circular in section, as in *Pteridium* and *Paesia*, and the receptacle is clearly conical in form. In these characters it maintains its similarity to the type of the less advanced Marginales.

These observations indicate what has probably been the phyletic history, that *Pteris* (*Histiopteris*) *incisa* has probably sprung from some Lindsayoid-Paesiod source with double indusium. The steps in advance were reticulation of the veins, formation of dermal scales, loss of inner indusium, and a tendency to slide the sorus on to the lower surface; this last comes out more clearly in the mature than in the young state. On the other hand, the anatomy is simpler than in *Pteridium*, but more complex than in *Paesia*. This has been already noted by

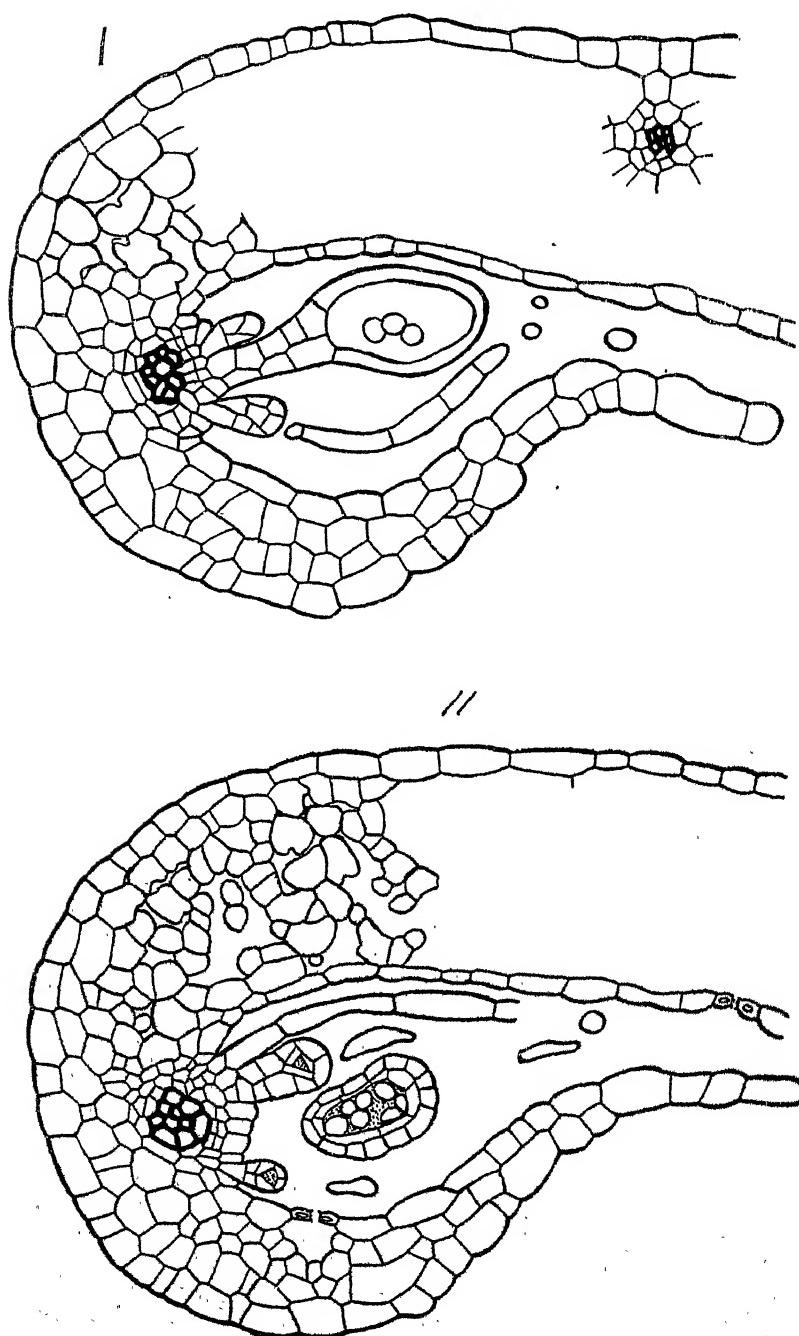


FIG. 25. i, ii. Vertical sections through more mature sori of *Histiopteris incisa*. ( $\times 75$ .)

Gwynne-Vaughan.<sup>1</sup> It illustrates what is often seen elsewhere, that anatomical and soral characters do not always march parallel.

This brings to a conclusion the study of selected examples of Prantl's Lonchitidinae, with which *Pteris (Histiopteris) incisa* (Thunbg.), J. Sm., has been associated very naturally. There is reason to believe that all of these Ferns are more or less related forms, as Prantl recognized them to be. The presence of hairs as dermal appendages, of solenostely, of a leaf-trace usually undivided, and of a marginal sorus, mostly with two indusial flaps, and an early indication of a gradate, passing over to a mixed sorus, are all signs of their relatively primitive position. Steps of advance are seen in one or another, in dermal scales (*Lonchitis*, *Histiopteris*), increasing disruption of the vascular tracts (*Pteridium*, *L. hirsuta*), medullary vascular developments in old *Histiopteris* and *Pteridium*, partial or complete loss of the lower indusium (*Histiopteris*, *Lonchitis*, *Paesia*, and even *Pteridium*), transfer of the receptacle from the true margin to the lower surface (*Histiopteris*). These and other features indicate advances to the border-line of the genus *Pteris*. That it is so is signalized by the frequent synonymy of the older writers. Thus the Lonchitidinae form the natural phyletic introduction to the condition shown in *Pteris*.

### *Pteris.*

Of the Pteridinae of Prantl<sup>2</sup> it is *Pteris* itself which is most clearly associated in habit, structure, sorus, and synonymy with the Lonchitidinae. The question then arises more directly for that genus than for the rest as to a probable phyletic relationship. It will therefore be taken first. Of the section §*Eupteris* the species *P. serrulata*, L. (= *P. multifida*, Poir.), *P. cretica*, L., *P. biaurita*, L., *P. longifolia*, L., and *P. heterophylla*, L. (= *Anopteris hexagona* (L.), C. Chr.), have been examined. Of the section §*Litobrochia*, in addition to *Histiopteris incisa* described above, *P. podophylla*, Sw., will be taken, and of the section *Heterophlebium*, *P. grandifolia*, L. The section *Doryopteris*, *P. sagittifolia*, Raddi, has been excluded by Prantl. This will for the present purpose be a sufficient representation. It must remain for others to carry out the detailed examinations which will be necessary to dispose the species of this large genus more exactly in phyletic sequences.

All these Ferns bear scales on the rhizome and leaf-base, not simply the primitive hairs. Though they vary a good deal in habit, they are all solenostelic, but with more or less marked advance towards dictyostely, while in some of them medullary complications of a high order are found. A few notes on their vascular anatomy will be given before proceeding to comparison of their sori.

<sup>1</sup> Ann. of Bot., vol. xvii, p. 735.

<sup>2</sup> I. c., p. 17.

## ANATOMY.

The fundamental type of the vascular system in the adult axis in the genus *Pteris* is solenostelic, with an undivided leaf-trace. This is seen very typically in *Pteris grandifolia*, L., and it has already been noted also in *Paesia scaberula* (A. Rich), Kuhn, and *P. viscosa*, St. Hill. These may all be held as retaining a prevalent, primitive construction.

But various modifications of this construction are found which may be held as derivative. The most common is the transition to dictyostely, resulting from the overlapping of the foliar gaps. This is noted by Gwynne-Vaughan<sup>1</sup> for *Pteris tremula*, R. Br., *P. cretica*, L., *P. flabellata* (? = *P. semipinnata*, Schkuhr), *P. (Anopteris) heterophylla*, Diels, and *P. pellucida*, Pr. To this list may now be added *P. swartziana*, Ag., and *P. biaurita*, L. In most of these the leaf-trace remains still undivided. But in some of them it comes away from the stele in the first instance as two separate straps. This is seen in *P. cretica*, L., and it matches very nearly, though on a much smaller scale, the condition seen in *Lonchitis hirsuta*, in *Gymnogramme japonica*, and indeed in many other Ferns of more remote affinity.

A more marked modification is that described at length by Gwynne-Vaughan,<sup>2</sup> viz. the appearance of accessory vascular strands. It is seen in *Pteris elata*, var. *Karsteniana*, Kze., now referred to *P. (Litobrochia) Kunzeana*, Ag., where the erect or oblique rhizome contains a perfect solenostelete, with a more or less complete internal vascular cylinder; this is connected by a compensation-strand with the outer cylinder, which attaches itself to it at the anterior margin of each leaf-gap. The indication how this polycyclic state may have arisen is given by comparison of the simpler condition seen in *Dennstaedtia adiantoides*<sup>3</sup> and *rubiginosa*.<sup>4</sup> This is by no means the extreme example of polycyclity in this affinity. *Saccoloma elegans*, Kl., has been described above. But this again is equalled or even exceeded by the complicated structure seen in the axis of *Pteris (Litobrochia) podophylla*, Sw. From material of this Fern collected in Jamaica it is possible to give a fairly complete account of the ontogeny of its very complicated stelar condition.

The petiole of juvenile leaves of *Pteris podophylla* shows a simple leaf-trace which takes a horseshoe shape in those successively of larger size. In adult leaves it still remains undivided, but the lateral folds of the horseshoe become deeply incurved, as in Fig. 26, a. This is the condition seen at the base of the petiole. But in large leaves, as the upper region is reached, the lateral folds may meet and fuse, while below the lowest pair of pinnae the fused adaxial curves of the horseshoe may separate completely from the

<sup>1</sup> I. c., p. 697.<sup>2</sup> I. c., Fig. 2.<sup>3</sup> I. c., p. 698.<sup>4</sup> I. c., Fig. 18.

abaxial part of the trace, which now forms a closed ring (Fig. 26, b). The behaviour of these vascular tracts in supplying the first pinnae and the extension of the rachis is shown in Fig. 26, c-i. Extending laterally the ring flattens, and two processes are formed on either of its flattened sides (c). These approach and meet, resolving the ring into three loops. Meanwhile similar fusions right and left of these re-establish connexion between the adaxial and abaxial tracts (d, e, f). Splittings which follow these fusions result in the establishment of three separate traces, supplying respectively the two pinnae and the continuation of the rachis. The former (g, i) have each a connected, but slightly unsymmetrical horseshoe; the latter repeats on a smaller scale the structure of the petiole (h). What has been described was seen in an individual case, and it is not known whether the relations are constant in leaves of large size in this species. Nothing exactly like this has been seen in other Ferns. There is, however, some distant resemblance to what has been described for large leaves of *Lophosoria* and *Gleichenia*; and analogous cases will probably be found in other large-leaved Ferns.<sup>1</sup> The nearest analogy, however, is with the condition of the petiole of *Acrostichum aureum*, L., as described by Frau Schumann.<sup>2</sup>

*Pteris podophylla* shows an extraordinary complexity of the vascular structure of its stem in large plants. It has already been noted by Mettenius, evidently in plants of medium size, for he compares it with what is seen in *Pteris elata*.<sup>3</sup> The material collected in Jamaica has served for tracing the development from the beginning to the fully adult state. Several young plants were cut serially, and parts of older plants, and from them the following account has been constructed, which shows that a much more complicated state is attained than in *P. elata*.

At the base is a protostele with parenchyma scattered among the tracheides. The parenchyma increases centrally to form a pith. At a comparatively early stage, and before phloem is present in quantity, this is converted into a solenostele by the process described as 'pocketing'. This structure is continued for several internodes in the usual way. But

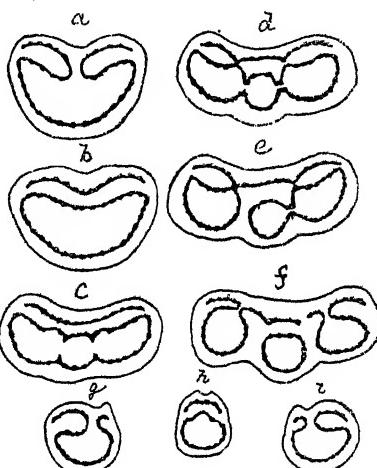


FIG. 26. a, b. Transverse sections of the petiole of *Pteris (Litobrochia) podophylla*; c-f show the steps of change to form the two first pinnae; g-i show the stalks of those pinnae, and of the rachis. Natural size.

<sup>1</sup> Compare Studies, II, Pl. XXXV, Figs. 14, 15.

<sup>2</sup> Flora, 1915, p. 212, Figs. 6, 7.

<sup>3</sup> I. c., p. 535.

incidentally it may happen that two pockets are observed side by side in the same transverse section, and pursue for some distance an independent course, finally coalescing. They thus register structurally the independent effect of the insertion of the several leaves upon the central tissues of the stele. Soon an encroachment of vascular tissue from the inner surface of

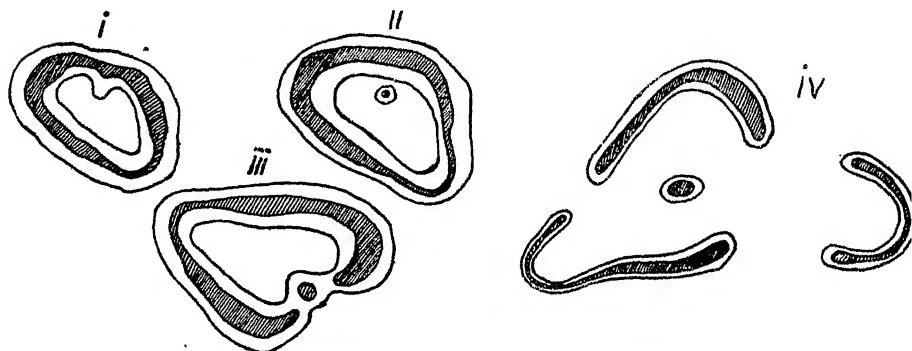


FIG. 27. i-iv. Vascular system of the young plant of *Pteris podophylla*, as seen in successive transverse sections at its base. ( $\times 35$ .)

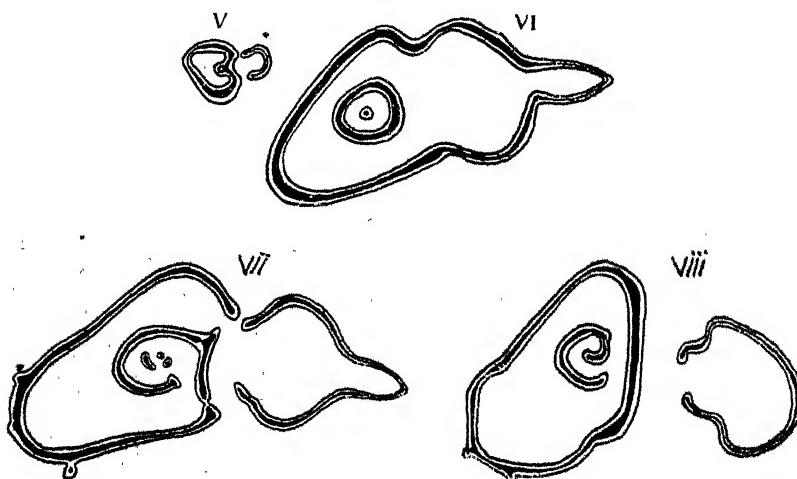


FIG. 27 bis. v-viii. Similar sections of *Pteris podophylla*, showing a more advanced state. ( $\times 5$ .)

the solenostele appears. It projects into the pith from the side of the tube where the last leaf-trace was given off (Fig. 27, i); it is abstracted (ii), and takes an oblique course across the tube to a point almost opposite, where meanwhile the next leaf-trace has just been detached (iii). There it acts as a compensation-strand filling up the leaf-gap. But almost at once

it separates again, moving across the pith to a point opposite the next leaf-trace, attaching itself as before on the basal lip of the leaf-gap. So far the leaf-gaps do not overlap. But a transition to dictyostely occasionally makes its appearance (iv), though this is not a common feature, and does not appear in the adult stem.

The next step is the expansion of the medullary strand into a second ring, which is brought about by a process of 'pocketing' similar to that seen in the original stele (Fig. 27 bis, v). It results in a second ring within the first. The further elaboration to form a second ring with a vascular strand again within it has not been actually observed; but there can be no doubt that it is a mere repetition of the earlier steps. The result is seen in vi, where there are two concentric solenosteles and a central strand. A leaf-trace is being formed from the outer, while the vascular extension at the extreme right supplies an abaxial bud which is formed at the base of the petiole, as in *Lophosoria* and other Ferns. In vii, which shows a section in a higher plane, the leaf-trace is detached, while a broad compensation-strand is forming from the inner ring to fill the leaf-gap. The single central strand has meanwhile branched. A section in a still higher plane (viii) shows the leaf-gap filled by the compensation-strand, while the central strands have fused to form a sort of secondary compensation-strand, and joined up with the inner solenostele.

As the stem grows larger, still greater elaboration of the vascular system may be seen. This is illustrated in Fig. 28, A-D, which shows the essential features of the very elaborate system, and the relation of the supply of axis and leaf. Three leaf-bases are included (*a*, *b*, *c*), and the drawings are in alphabetical order from below upwards. They demonstrate the steps in giving off the trace *a*. In Fig. 28, A, wide dilatations of the outer unbroken solenostele are shown corresponding to the leaf-bases *a*, *b*, and a smaller one for leaf *c*. The inner ring is also complete, while within it a somewhat irregularly interrupted third ring is seen, with two central vascular strands. B shows the formation of the leaf-trace *a* more advanced, while the compensation-strand which is to fill its leaf-gap is partly detached from the second solenostele. A similar bar of vascular tissue parallel to the first compensation-strand is being formed from the third ring. In section C the leaf-trace is detached, and forms a completely closed, though fluted ring. After what has been seen in the petiole such an occurrence will not cause surprise; but it has only been observed in fully adult leaves. Section C shows that the outer solenostele is completed by the fusion of the compensation-strand with the margins of the leaf-gap, while a similar replacement has taken place so as almost to complete the second solenostele. The third ring and central strands are here irregularly interrupted. But in section D they have again settled down to constitute a third, almost complete solenostele, and central vascular core. The second and outermost

solenosteles are complete, while the latter is beginning to show the incurving to form the leaf-gap for the departure of leaf  $b$ .<sup>1</sup>

It is thus apparent that the vascular system of a large stem may form four concentric tracts; but that the direct vascular connexions of the leaf are with the outermost ring only; while secondary connexions with the inner solenosteles copy with less regularity the behaviour of the outermost.

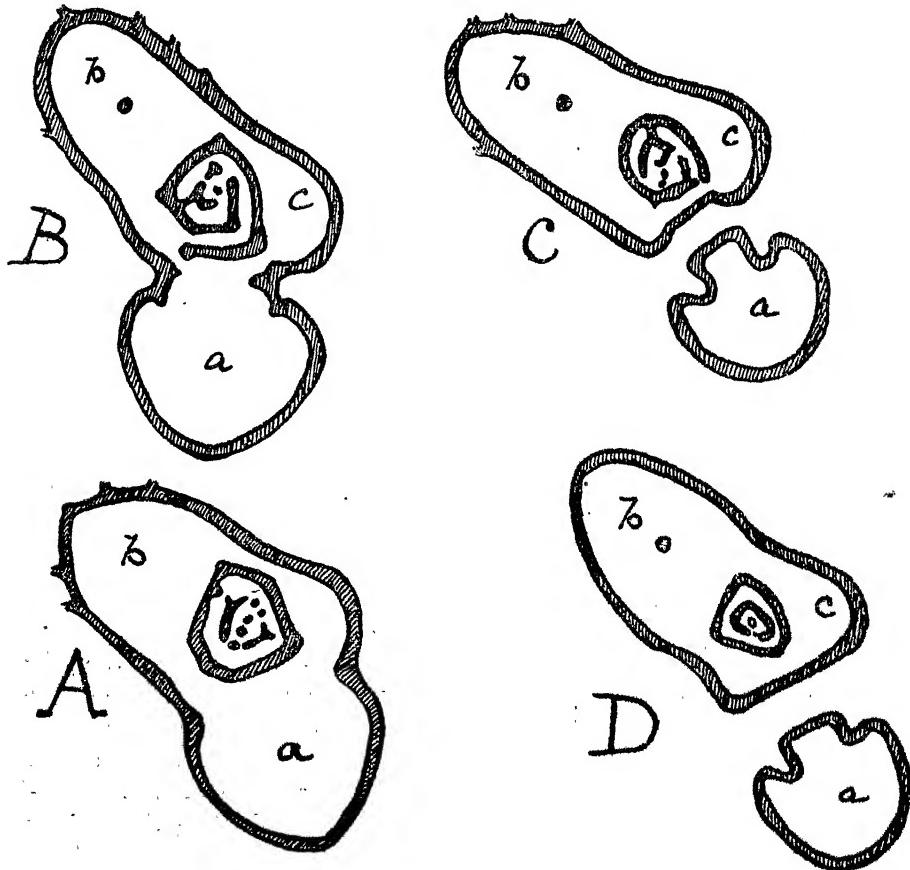


FIG. 28. A-D. Vascular system of an old plant of *Pteris podophylla* seen in transverse sections successively from below upwards. Natural size.

The irregularity is greatest in the innermost tracts of vascular tissue. Compared with other elaborate vascular arrangements the general dis-

<sup>1</sup> It is worthy of note that an isolated vascular strand makes its appearance in the dilated base of the leaf  $b$ . It is shown in the sections B, C, D, but it is occasional, and appears only in large leaves. It is entirely disconnected with any other vascular tract, upwards or downwards. In fact it is a detached island of vascular tissue. Yet it consists of a xylem-core, an outer band of phloem and conjunctive parenchyma, and an endodermis. This is a good example of the formation of tissues, and especially of endodermis, *de novo*, and it is worthy of the attention of those who attach importance to the continuity of such tissues in morphological argument.

position in *Pteris podophylla* resembles that in *Pteris elata*, in *Saccoloma*, in *Acrostichum aureum*, and in *Thyrsopteris*. The marginal series thus serves to provide the most striking examples. There are differences of detail between all of these cases. It is not probable that any of these Ferns are in so close a phyletic relation as to rule out the view that they represent independent elaborations under substantially similar conditions. If that were their true origin, the differences of detail would be readily understood.

#### SORUS OF *PTERIS*.

It has been seen in *Histiopteris incisa* that the inner (abaxial) indusium is absent, and that the reference of the receptacle to a strictly marginal origin is dubious. It was suggested that this illustrates the result of a phyletic slide of the sorus from the actual margin, as in *Lindsaya* and *Paesia*, on to the lower surface. In *Pteris* that superficial origin of the sorus is constant, and it has become the generic character. The inner indusium is regularly absent, and the persistent upper or outer (adaxial) indusium corresponds now not only in appearance, but also in origin, to the margin of the leaf. In fact, the phyletic slide of the originally marginal sorus to the surface is complete. The sorus thus constituted, covered in by the adaxial indusium and served by an underlying vascular commissure, shows in the different species a considerable latitude of construction. Selected species illustrating this will be described in succession, taking those with the simplest sorus first.

In *Pteris longifolia*, L., the segmentation of the young pinna is of the usual marginal type (Fig. 29, *a*). The first sporangium makes its appearance upon the concave (abaxial) surface, distinctly within the margin but near to it (*b*). The margin itself develops into the indusium, while there is no indication of the inner indusium. Later the vascular commissure appears as a compact strand underlying the narrow, slightly convex receptacle. The upper indusium becomes much extended as a very efficient protective flap. The sorus is mixed, but there are relatively few sporangia intermingled with paraphyses.

*Anopteris hexagona* (L.), C. Chr. (= *Pteris heterophylla*, L.), has been brought into special prominence by Mettenius<sup>1</sup> in relation to his theory of connation (*Verwachsung*) of hairs to form the inner indusium. He states for this species that the ‘paraphyses’ are disposed in a series along the inner limit of the sorus, and are so closely ranged that they seem to form an indusium which grows out distally into hairs projecting as cilia upon its margin. His Fig. 17 shows the natural position of these hairs. He concludes (p. 7) that the inner indusium (*Schleier*), such as in *Pteridium*,

<sup>1</sup> Farngattungen, iii, ‘Ueber die mit einem Schleier versehenen Arten von *Pteris*’, p. 6. Frankfurt, 1858.

has arisen by fusion (*connatus*) of hairs such as are seen in *Pteris heterophylla*.

Naturally the latter species had to be examined and the truth of this observation tested. This was possible on material collected in Jamaica. It was found in sections cut vertically through the young sorus that the hairs are very numerous, but always distinct from one

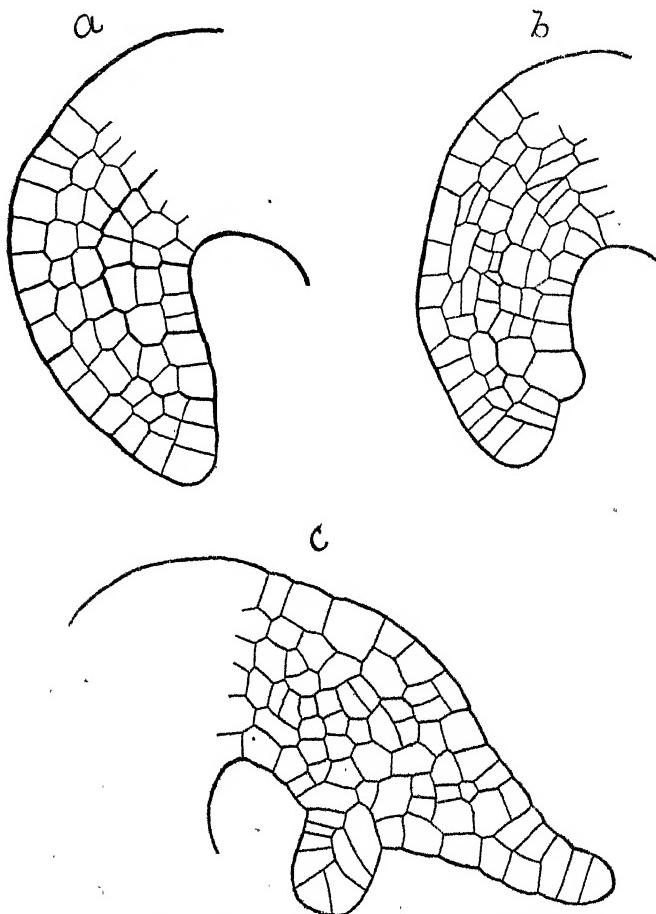


FIG. 29. a-c. Vertical sections of the young sorus of *Pteris longifolia*. ( $\times 150$ )

another, never webbed. The sporangia are relatively few. The position in which the first hairs (paraphyses) originate has no definite relation to the inner limit of the receptacle, where the inner indusium should be theoretically. They are sometimes found singly on the side next the upper or outer indusium (Fig. 30, a); sometimes in a close group, covering about half the width of the receptacle (b). In rather older states

the whole width of the receptacle, as seen in vertical section, may be occupied by them (*c*). Where a sporangium is included in the section the hairs may be on either side of it (*d*). Now these facts do not fit with Mettenius's theory of the origin of the inner indusium by a fusion of hairs—a theory which is quite out of harmony with the comparisons which are being developed in this memoir. Using the method of Mettenius it would be equally possible to suggest that the hairs seen in Fig. 30, *c*, were in course of formation of a *transverse* indusium! But what is fatal is that there is no constancy of the hairs in the position where the inner indusium should be: nor has any webbing been observed in them. It is not intended

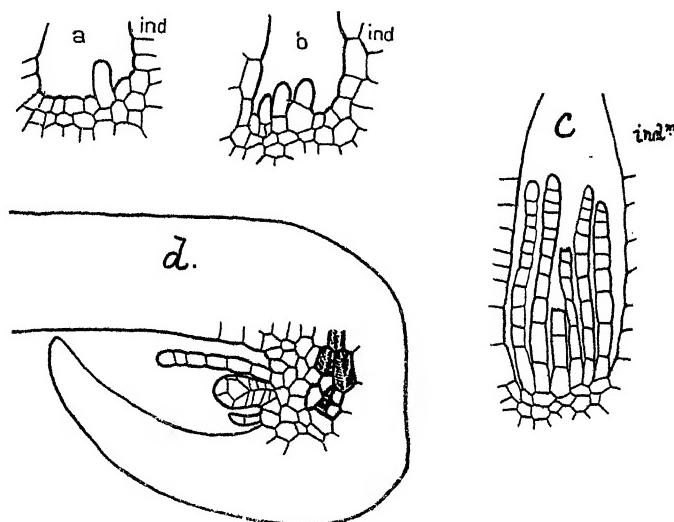


FIG. 30. *a-c.* Sections through the young sorus of *Anopteris hexagona* (L.), C. Chr. (= *Pteris heterophylla*, L.), showing the origin and relation of the soral hairs. (*a, b*,  $\times 125$ ; *c*,  $\times 85$ .)

to deny that any of the hairs can be held as representing an inner indusium. But if any of the hairs are of that nature phyletically, they represent not an inner indusium in the making, as Mettenius suggested, but the degenerate relics of an inner indusium which, being of no further use, is in course of dissolution. The view which I take, however, is that the hairs seen in the sorus of *Anopteris hexagona* are simply paraphyses scattered over the receptacle, as they are in other species of *Pteris*; though here they are more closely arranged than usual.

In *Pteris serrulata*, L. fil. (= *P. multifida*, Poir.), the sporangia are again of intramarginal origin, and are produced almost simultaneously in considerable numbers (Fig. 31, *a*); but the mixed character of the sorus becomes almost at once apparent (*b*). The segmentation clearly shows that it is the actual margin which grows directly into the protecting

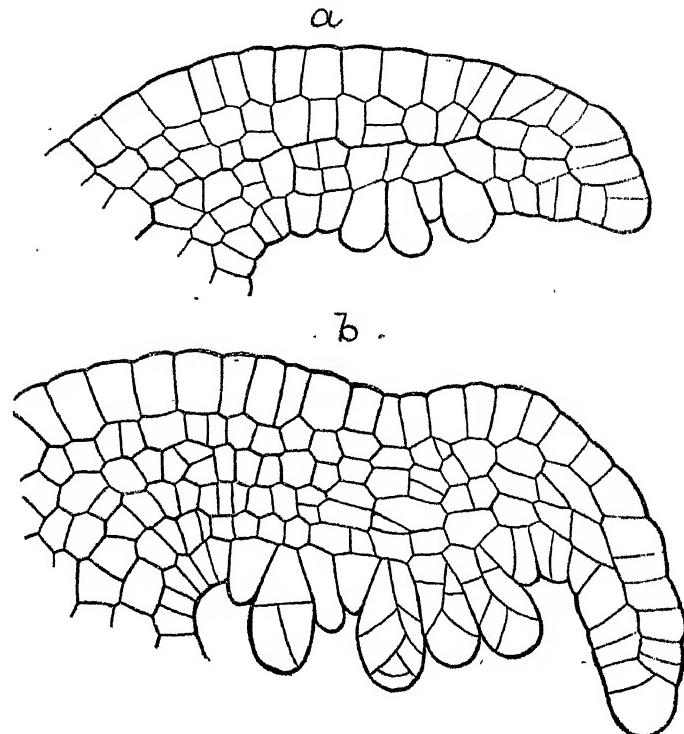


FIG. 31. *a, b.* Vertical sections of young sori of *Pteris serrulata*. ( $\times 15.$ )

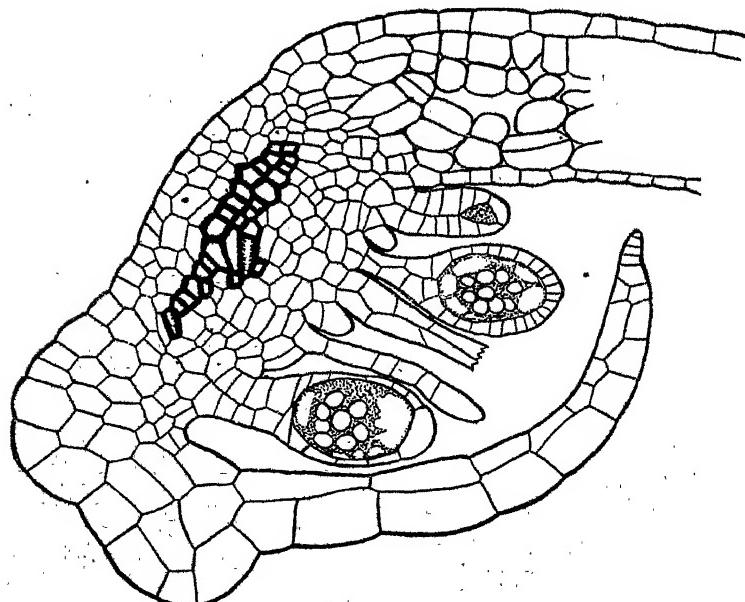


FIG. 32. Vertical section of advanced sorus of *Pteris cretica*. ( $\times 75.$ )

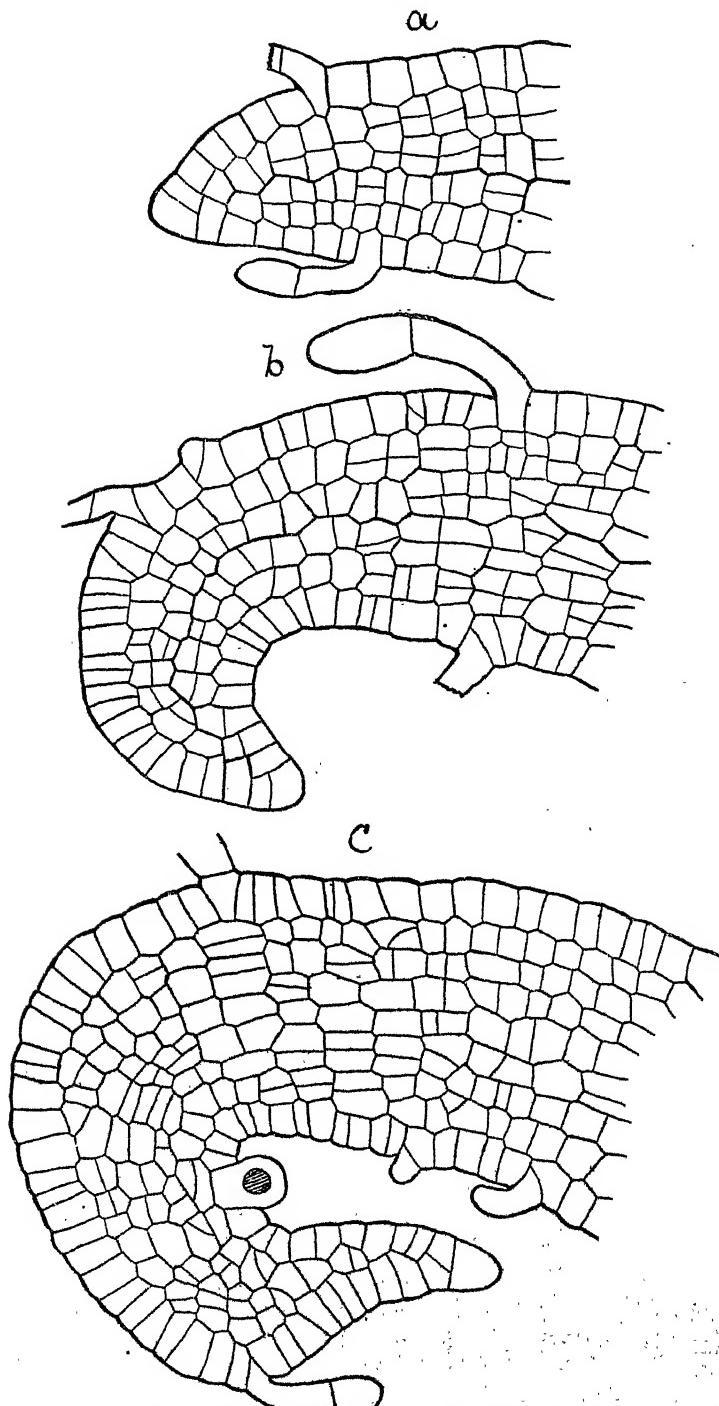


FIG. 33. *a-c.* Successively older sori of *Pieris quadriaurita*, seen in vertical section. ( $\times 150$ .)

flap. When mature the sorus appears in section as a considerable area of leaf-surface curved downwards, and with the margin running out into the not highly specialized indusial flap.

A rather more advanced condition is seen in the related species, *P. cretica*, L. (Fig. 32). Here the general relations are the same. The receptacle, which is broad as in *P. scrrulata*, is only slightly convex. Below it lies a widely expanded commissure of trachcides suited to supply the large area of the receptacle, upon which the numerous sporangia of the mixed sorus are borne. It will be noted that the oldest sporangium

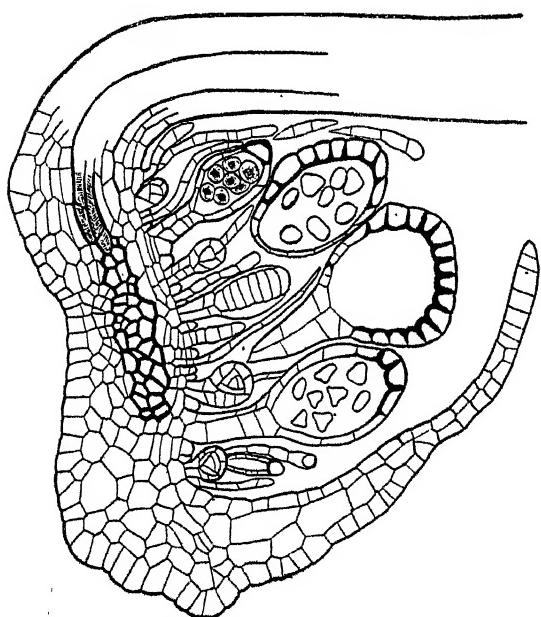


FIG. 34. Vertical section of the mature sorus of *Pteris podophylla*. ( $\times 85$ .)

advance in specialization upon those of *Paesia*, *Pteridium*, and *Lonchitis*.

The specialization is more clearly shown in *P. biaurita*, L., where again the indusial flap is directly marginal (Fig. 33, *a*, *b*). It is to be noted that the origin of the first sporangium is delayed until the foundation of the massive sorus is fully laid (*c*). But this point is still more strikingly illustrated in *P. podophylla*, Sw., material of which was received from Jamaica through the kindness of Mr. Harris. The earliest stages were not observed, but the mature sorus shows a very wide, almost flat receptacle, with extended vascular commissure (Fig. 34). The sporangia are very numerous, with various ages intermixed, and numerous hairs distributed throughout.

*Acrostichum*, L. (1737).

It is thus seen that the fusion-sorus may be considerably widened in certain species of *Pteris*, of which the *Pteris*-nature has never been in doubt. These may be held to prefigure other cases in which the *Pteris*-affinity is less obvious. One of them is the Fern designated *Acrostichum praestantissimum*, Bory, which is closely related to *Acrostichum aureum*, L. It is a very local West Indian Fern, figured in Hooker's 'Garden Ferns', Plate 58. Its habit is rather coarse, with a thick upright stock, simply pinnate leaves, and a reticulate venation of the *Litobrochia*-type. This has suggested a comparison especially with *P. (Litobrochia) splendens*. The leaves are dimorphic, and many specimens show in the fertile leaves the whole lower surface covered with sporangia, as in *A. aureum*. But in other specimens the sorus extends only part way from the margin to the midrib. This was noted by Féé, who is quoted on the point by Sir W. Hooker. The latter remarks that 'there is in that state a distinct narrow continuous involucrum, as in *Pteris*, closely covering the sori'; and it is shown in his Fig. 3, Pl. 58. This comparison has recently been revived by Frau Schumann,<sup>1</sup> who indicates specially the relation to *P. splendens*, and has added many facts bearing on the comparison.

Conditions linking the fully Acrostichoid state with the *Pteris*-type of sorus may be found by examining pinnae of *A. praestantissimum* from the apex downwards. It is held on grounds of anatomy, and also of general morphological comparison, that the apex and base of the leaf show relatively primitive features, while the middle region is phylogenetically advanced as compared with them. If this be true, a basipetal sequence of sections from the tip would progress from the more primitive to the derivative region. Small pieces of herbarium material were available from Kew and the British Museum, and serial sections showed the essential points

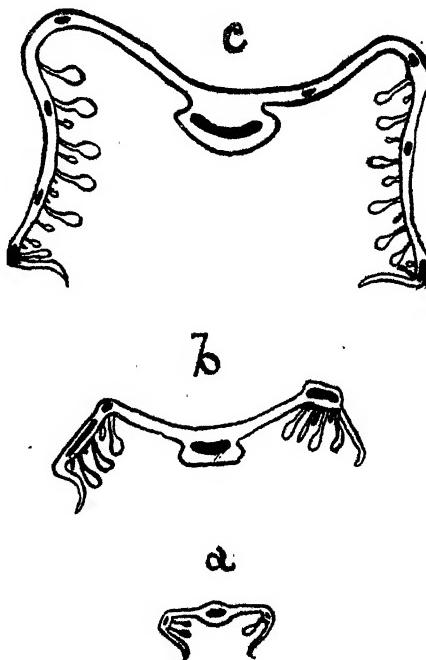


FIG. 35. a-c. Successive sections of a pinna of *Acrostichum praestantissimum*, from the apex downwards, showing widening of the Pterid sorus. ( $\times 20$ .)

<sup>1</sup> Flora, 1915, pp. 220, 243.

required. A specimen of a young pinna of a narrow type from Martinique, collected by Hahn, 1868, showed at the distal end a purely *Pteris*-structure (Fig. 35, *a*), with well-marked indusial flap, a narrow receptacle traversed by a vascular commissure, and the relatively few sporangia strictly limited in their insertion to the receptacle. Lower down the receptacle itself widens (*b*), and the sporangia are at first still restricted to it; but lower down they encroach in their insertion upon the free surface of the pinna (*c*). This may extend in broader-leaved examples fully half-way to the midrib (Fig. 36); or indeed the whole distance, as in Hooker's Plate 58. It will be noted from the surface view that the indusium is continuous, and destitute of vascular tissue, and that the vascular commissure, which is continuous, is considerably widened, but that the soral area extends far beyond it. Thus it is not merely a widening of the receptacle, as in *P. podophylla*, but an actual overflow on to the leaf-surface.

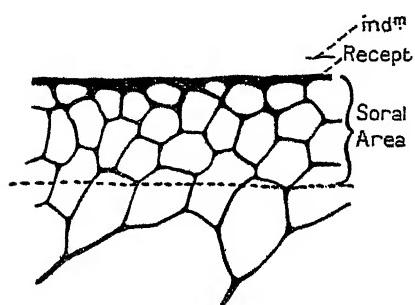


FIG. 36. Margin of a fertile pinna of *Acrostichum praestantissimum*, showing in surface view the indusium, receptacle, and soral area. ( $\times 10$ .)

Such comparisons are obviously insufficient without anatomical and other evidence. Unfortunately it is improbable that material of *A. praestantissimum* will be available at present. *A. aureum* has been examined by Miss Thomas<sup>1</sup> and by Frau Schumann.<sup>2</sup> Neither of these accounts is wholly satisfactory, and the matter is being reinvestigated by Dr. Thompson, with a view to comparisons with such types as *Saccoloma* and *P. podophylla*.

#### CONCLUSIONS RESPECTING THE GENUS *PTERIS*.

The genus *Pteris*, in the old comprehensive sense of the 'Synopsis Filicum', included a number of sub-genera. Some of these are now distinguished as substantive genera, as indeed they had been previously by certain writers. They, together with the genera *Lonchitis* and *Actiniopteris*, are associated in Christensen's Index (pp. xliii–xliv) under 'VI. 4. Pteridinae'. With them should now be associated phyletically the genus *Acrostichum* in the original sense of Linnaeus, now adopted by Christensen (l.c., p. 4). Possibly some others will be added when the details necessary for phyletic conclusions have been sufficiently worked out. These Ferns appear to constitute a natural group, which probably have had a common ancestry, though this was not recognized by the older systematists who classified them together. They have probably all originated from an

<sup>1</sup> New Phyt., vol. iv, 1905.

<sup>2</sup> Flora, 1915, p. 208, &c.

intermediate type with marginal sorus, protected by indusial flaps on either side. Comparison of them *inter se* indicates that such a type would have had hairs as dermal appendages, a relatively simple vascular system, an isolated gradate sorus with convex receptacle, and sporangia with a complete, oblique annulus, a lateral dehiscence, and a number of spores not exceeding 64.

This type probably sprang ultimately from some Schizaeoid source, in which as in *Lygodium* the vascular system was quite primitive, the dermal appendages hairs, and the sporangia actually marginal. Sorally the sequence probably proceeded, through characters now exemplified by the Dicksonioid and Lindsayoid types, to the ultimate Pterid characters. It is not suggested that the species now constituting such genera were themselves the progenitors of the Pterids, but that they illustrate their probable phyletic origin. There would thus have been a transition from the isolated marginal sorus, with double indusium and marginal receptacle, as seen in *Dicksonia*, to the superficial fusion-sorus, with vascular commissure and a single indusium. This would have involved not only a lateral fusion of sori, but also a 'phyletic slide' of the receptacle to a superficial position, in which case, as the sorus becomes deflected to the lower surface, the inner (abaxial) indusium becomes non-functional and obsolete. This transition has been illustrated developmentally in various species, but in none so convincingly as in *Histiopteris incisa*, which is linked so directly with *Pteridium* by similarity of habit. It thus appears that the genus *Pteris* as at present defined by Christensen, together with *Histiopteris* and *Lonchitis*, has arrived at the present uni-indusiate state by loss of the inner indusium, which is still present in *Pteridium* and *Paesia*. It may, however, turn out later that some species now ranked as *Pteris* may have to be referred to some other source. Till decisive evidence is available, the usual systematic grouping of the species should be followed; and the conclusion may accordingly apply provisionally to the whole genus as accepted by Christensen.

Once the inner indusium is obsolete, the fusion-sorus may widen its receptacle, or it may extend inwards. Various degrees of widening of the receptacle may be seen, and an extreme case has been demonstrated in *Pteris podophylla*. Or it may extend towards the midrib, covering the surface in part (*Acrostichum praestantissimum*) or wholly (*A. aureum*, L.). On the other hand, the converse progression is illustrated by *Dictyoxiphium*, in which, as has been shown above, the upper indusium is abortive, and the receptacle is tilted towards the upper surface, with some indication of its spreading upon that area, somewhat after the manner of *Acrostichum praestantissimum* upon the lower. Thus along steps individually slight, states may be attained apparently very different from the initial condition of the isolated, marginal, bilobate sorus of the Dicksonioid type.

*Hypolepis*, Bernh.

The fact now shown that either the upper indusium (*Dictyoxiphium*) or the lower (*Pteris*) of the two-lipped sorus may be abortive, raises the question whether in any other genera systematically associated with the Pterideae there is reason to believe that a similar abortion of the indusium has occurred. It seems probable that *Hypolepis* may best be interpreted in this way. This genus has been variously treated by systematists. Presl<sup>1</sup> places it with *Lonchitis*, and close to *Cheilanthes*. Hooker<sup>2</sup> assigns to it a similar place. Christ<sup>3</sup> associates it with *Phegopteris*, and especially with *Phegopteris punctata* (Thunbg.), Bedd. Diels places it in his Pterideae-Cheilanthinae, in near relation to *Cheilanthes* and *Llavea*,<sup>4</sup> and in this Christensen follows him.<sup>5</sup> In all of these decisions it appears that the chief weight of the comparison has been laid upon the sorus, with its single, marginal indusium, and its usually subglobose form.

But Sir William Hooker<sup>6</sup> discusses the habit of these plants also, as bearing upon their systematic position. He points out how Presl in his 'Tentamen' limits the genus to those species corresponding to the *Microlepia* group of *Dicksonia*, and that John Smith adopted the same view. The latter observes that 'this genus is formed of a group of species characterized by their large decomound fronds, which arise from a lengthened creeping rhizoma similar in habit to some of the large-fronded species of *Polypodium*,<sup>7</sup> and differing from them only in the soriferous crenule being altered in texture and reflexed, and therefore not distinct in that respect from *Cheilanthes*; but their whole habit naturally indicates them to be a distinct group from the species which I retain as true *Cheilanthes*'. If those who followed had given more attention to the habit-characters, *Hypolepis* might have sooner arrived at some more fixed systematic position. Evidently Diels was dissatisfied with its being placed among the Cheilanthinae, for he remarks<sup>8</sup> that *Hypolepis* has in habit little in common with the other types of this series. To us, who now realize that a very similar soral structure may arise along distinct phyletic lines, the habit-character acquires additional value. In the present case it is possible that the sorus of *Hypolepis* may have come from some Dicksonioid-Davallioid source, by abortion of the inner indusium, while that of *Cheilanthes* may have been uni-indusiate from the first. In which case *Hypolepis* would be detached from the superficial comparison with *Cheilanthes*. Already Kuhn (1882), and later Prantl (1892), had placed *Hypolepis* with *Dennstaedtia*, *Microlepia*, *Leptolepia*, and less certainly *Saccoloma*, in the newly erected group of the

<sup>1</sup> Tentamen, p. 161.<sup>2</sup> Syn. Fil., p. 128.<sup>3</sup> Familiäuter, p. 278.<sup>4</sup> l. c., p. 277.<sup>5</sup> Index, p. xlvi.<sup>6</sup> Sp. Fil., vol. ii, p. 59.<sup>7</sup> This is clearly an allusion to types such as *Polypodium punctatum*, Thunbg., the relation to which will be considered later.<sup>8</sup> l. c., p. 277.

Dennstaedtiinae, which is probably its natural place;<sup>1</sup> though Prantl regarded the position assigned for *Hypolepis* as provisional,<sup>2</sup> since he had no developmental material at hand. It will therefore be useful to re-examine the characters upon which the genus is so placed.

The similarity of character of *Dennstaedtia* and *Hypolepis* depends first on habit: the creeping rhizome, with relatively long internodes, and ample upright-growing leaves of high pinnation and finely cut; the vestiture of hairs only; and the numerous sori, each seated in a sinus of the margin. Anatomically the rhizomes in both are solenostelic. Gwynne-Vaughan has shown the similarity of the vascular system of *Dennstaedtia punctiloba* and *apiifolia*<sup>3</sup> to those of *Hypolepis millefolia*, *tenuifolia*, and *repens*.<sup>4</sup> Though the latter show some advance in detail on the former, still they are less complex than *D. adiantoides*<sup>5</sup> or *rubiginosa*,<sup>6</sup> in which a medullary system exists. The leaf-trace also comes off undivided, though interruptions of its continuity, of the nature of perforations, may occur above their base, as they do also in the solenosteles of some of them. But these details do not materially affect the substantial likeness. The chief distinction lies in the sorus. That of *Dennstaedtia* is cup-like, and only indistinctly two-lipped, while that of *Hypolepis*, though corresponding in position, has only a single lip, viz. the upper or adaxial, which curves more or less over the receptacle, and is rather membranous in texture. It has been described as 'formed out of the reflexed margin'.<sup>7</sup> The converse of this is probably correct: viz. that it has become the equivalent of the margin, though phyletically it is a superficial growth.

In his excellent analysis of the Hay-scented Fern, *Dennstaedtia punctilobula* (Michx.), More,<sup>8</sup> Prof. Conard has already shown the receptacle and the first sporangium to be truly marginal in origin. But in order to establish our own comparisons, drawings have been made on the one hand of sori of *Dennstaedtia dissecta* (Sw.), Moore, and on the other of *Hypolepis nigrescens*, Hk., and *repens* (L.), Pr., all from specimens collected in Jamaica. Fig. 37, *a*, shows in outline from below a pinnae of *D. dissecta*, with its cup-like sori on the anadromic branches of the veins. The vascular strand terminates in the receptacle as an expanded mass of tracheides. The receptacle is marginal in origin, and the indusial flaps are consequently superficial.<sup>9</sup> Their relation is shown in Fig. 37, *b*, and it is there seen that the actual margin bears the first sporangium, while a second less advanced is already initiated below, thus indicating a basipetal sequence. This was found to be the rule in *Microlepia*, and it is seen in *D. apiifolia*;<sup>10</sup> but it has been shown that in *D. rubiginosa* the basipetal succession is

<sup>1</sup> Arb. K. Bot. Gart. Breslau, I. i, p. 16.

<sup>2</sup> l. c., p. 19.

<sup>3</sup> Solenostelic Ferns, II. Ann. of Bot., 1903, Pl. XXX, Figs. 1, 2.

<sup>4</sup> l. c., Figs. 4, 5, 6.

<sup>5</sup> l. c., Fig. 2.

<sup>6</sup> l. c., Fig. 13.

<sup>7</sup> Carnegie Institution, Publication No. 94, Figs. 151, 155, 158.

<sup>8</sup> Syn. Fil., p. 128.

<sup>9</sup> Cf. Conard, l. c., Fig. 151.

<sup>10</sup> Land Flora, Figs. 332, 332 bis.

departed from, and that is the case also in *D. dissecta*. Fig. 37, c, represents in outline the sorus of the latter in a more mature state. The indusial lips gape widely; the flattened, but still convex, receptacle bears

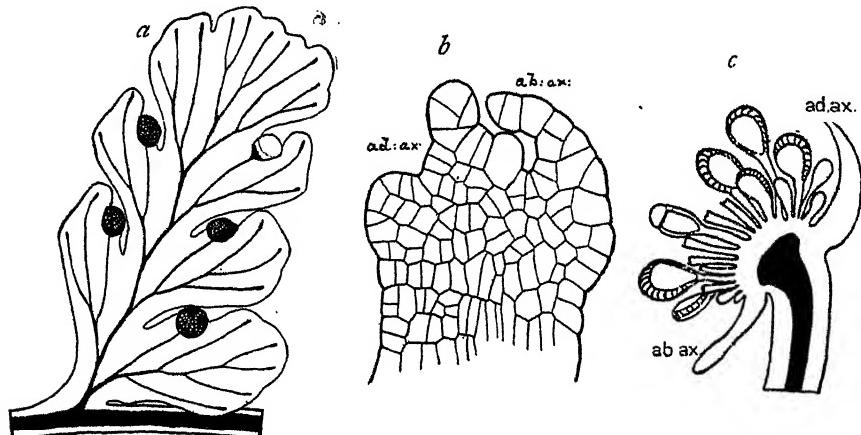


FIG. 37. a. Pinnule of *Dennstaedtia dissecta* seen in surface view, showing marginal sori on the apex of the anadromic branches of the veins. ( $\times 4.$ ) b. Sori very young, cut in vertical section, showing the marginal receptacle and superficial indusial flaps. ( $\times 150.$ ) c. Mature sorus in vertical section. ( $\times 35.$ )

numerous sporangia, and the stalks of others already dehisced. The latter are mostly near the centre, while younger sporangia are nearer the margins. Thus there is a general indication of a basipetal sequence, but the mixed character becomes obvious with age. The facts indicate that *Dennstaedtia*,

and in a less degree *Microlepia*, are transitional between a gradate and a mixed condition, but the relation downwards is clearly to the Dicksonieae, with which relation the anatomy and dermal hairs will also coincide.

A comparison of the sorus of *Hypolepis nigrescens* shows in section a very similar outline (Fig. 38); but the curvature to the lower surface is stronger, the sorus is more definitely of the mixed type, while the generic character is the absence of the inner (abaxial) indusium. It may readily be interpreted as a more advanced derivative, the absence of the inner indusium being related to its protection being no longer needed, where the curvature is strong. But *Hypolepis repens* differs in certain details from *H. nigrescens*. In some

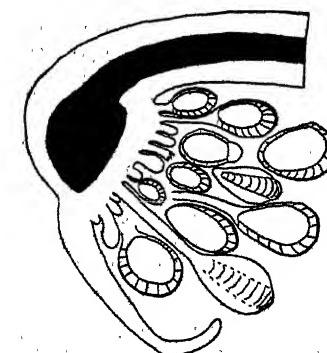


FIG. 38. Mature sorus of *Hypolepis nigrescens* cut vertically, showing its mixed character and the absence of the inner indusium. ( $\times 85.$ )

protection being no longer needed, where the curvature is strong. But *Hypolepis repens* differs in certain details from *H. nigrescens*. In some

examples of this species the indusial flap is reflexed;<sup>1</sup> but in others it may be found expanded in the plane of the pinnule. This state is shown in Fig. 39, which was drawn from a specimen brought from Jamaica as *H. repens*, though it may be one of those transitional forms to *Polyodium punctatum* which have been noted by so many collectors. Both the species named are native in Jamaica. It will be noted that the sorus is considerably extended along the vein, and that the latter, instead of terminating at the receptacle, extends onwards for an appreciable distance into the rounded marginal lobe, which thus represents the upper (adaxial) indusium. It is this lobe which in typical forms of *H. repens* curves over and protects the

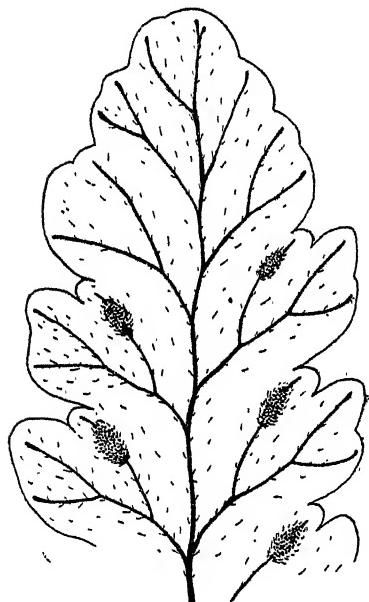


FIG. 39. Pinnule of *Hypolepis repens* seen in surface view. ( $\times 10$ .)

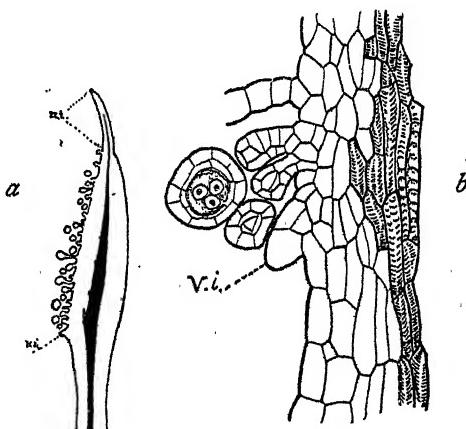


FIG. 40. *a.* Young sorus of *Hypolepis repens* cut vertically. *u.i.* = upper indusium, traversed by a vascular strand; *v.i.* = vestigial lower indusium. ( $\times 15$ ) *b.* Small part of the soral surface including the vestigial indusium (*v.i.*), more highly magnified. ( $\times 160$ ).

sorus. The extension of the vein into the indusial flap will be important for comparison with other cases, for it plainly discounts the value of the relation of the sorus to the vein-ending.<sup>2</sup>

A vertical section through such a sorus, following the vein (Fig. 40), shows how greatly the receptacle is extended and flattened, while the vein is seen continuous far into the distal indusial flap, upon which sometimes isolated sporangia and hairs may be scattered for some distance upwards. If in presence of these palpable differences from *H. nigrescens* any doubt were felt of the relation of this form to *H. nigrescens*, and ultimately to

<sup>1</sup> Cf. Hooker, Sp. Fil., vol. ii, Pl. XC, B.

<sup>2</sup> Compare the extension of two vascular strands into the indusial cup in *Davallia dissecta*, Goebel, Flora, Bd. cv, 1912, p. 47, Fig. II.

*Dennstaedtia*, the fact that a vestigial lower indusium may sometimes be found would remove it. The small projection (*v.i.*) at the lower limit of the receptacle in Fig. 40, *a*, is in the position of the lower indusium, and it is held to be its vestigial representative. It is shown under a higher power in Fig. 40, *b*. In most cases, however, the inner indusium is altogether absent, while the receptacle is often more compact and circular. In this condition the sorus appears superficial upon a vein, and distinctly intra-marginal. This is the characteristic of the Fern now designated in Christensen's Index as *Dryopteris (P.) punctata* (Thunbg.), C. Chr. It has been variously ascribed to *Polypodium*, *Phegopteris*, *Hypolepis*, *Nephrodium*, &c. Many authors have remarked how impossible it is to draw a definite line between it and species of *Hypolepis*.<sup>1</sup> In particular Sir Joseph Hooker remarks on the genus *Hypolepis* in the 'Flora Tasmaniae'<sup>2</sup> that 'sometimes the reflexion of the pinnules' margin is so slight that the sorus is really naked, and then I cannot distinguish the genus from *Polypodium*, or the species *H. tenuifolia* from *P. rugulosum*, Lab.' And later, referring to *H. tenuifolia*, he says: 'There is a New Zealand variety of this species with nearly glabrous rachis and stipes, more distant acuminate secondary and tertiary pinnae, narrow pinnules which are deeper lobed, and bear more numerous sori, scarcely covered by the involucre, and which hence passes into *Polypodium rugulosum*, Lab.' Mr. H. Carse, comparing such types on the spot in New Zealand, remarks:<sup>3</sup> 'In some forms of *Hypolepis* the spurious involucre (= inner, or lower indusium) is hardly or not at all developed, and sometimes it appears slightly in *Polypodium*, while the sori of the latter are frequently distinctly marginal.' Thus he had already noted the vestigial indusium.

The fact then appears to be that these Ferns are individually variable in the form and position of their sori. There is no obligation to draw any line between them. That they are very closely related is shown by their habit-similarity, by the absence of scales and presence of simple hairs, and by the practically identical vascular structure, as noted by Gwynne-Vaughan.<sup>4</sup> We may take it that in this nearly related series, *Hypolepis* has been the designation of those forms which are more conservative of the Dennstaedtioid characters, while the more advanced forms have been referred to *Phegopteris* or *Polypodium*.

A phyletic sequence may be traced thus. Starting from a Dennstaedtioid-Dicksonioid source, with marginal sorus, a two-lipped indusium, receptacle conical, gradate sequence of sporangia, having oblique continuous annulus, the sorus becomes 'mixed', the receptacle flattened, and curved downwards;

<sup>1</sup> Sir W. Hooker, Sp. Fil., vol. iv, p. 273; Syn. Fil., pp. 130, 312; Griesbach, Flora Brit. W. Ind., p. 67; Bentham, Flora Austr., vii, p. 765.

<sup>2</sup> Vol. ii, p. 138.

<sup>3</sup> Trans. N.Z. Inst., vol. xlvii, p. 85.

<sup>4</sup> Ann. of Bot., vol. xvii, p. 694.

the lower indusium reduced and abortive; the upper flattened out, and assimilated to the laminar surface, while it is traversed by a vascular strand extended from the receptacle. But it is not phyletically a lobe of the lamina, however nearly it may resemble one. It is originally a superficial indusial growth, as in the Dicksonioid Ferns. All of the Ferns involved in this series are rhizomatous and solenostelic, with united leaf-trace; bearing hairs, not scales; and they have highly ramified leaves, with open venation. A '*Polypodium*' type is thus seen to arise by modification of a Dicksonioid type. It is, however, to be noted that if the phylesis here suggested is correct, *Polypodium punctatum* will have no relation by descent with *Phegopteris* or *Dryopteris*, which names have been applied to non-indusiod Nephrodioid Ferns. The absence of scales and the solenostelic structure of

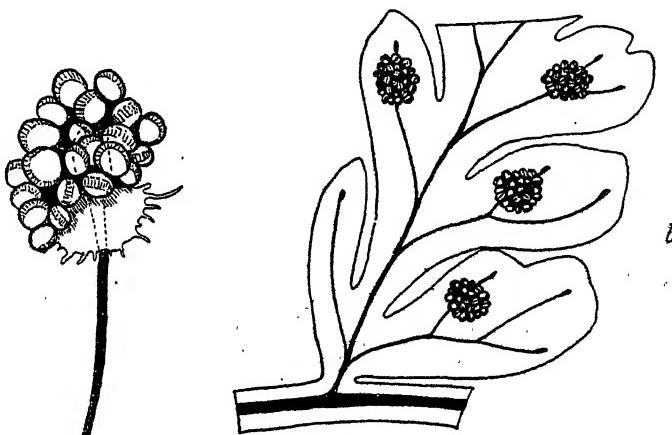


FIG. 41. a. Sorus of *Acrophorus nodosus* in surface view. ( $\times 35$ .) b. Pinnule of the same, showing the vein continued beyond the sorus. ( $\times 10$ .)

our series is in strong antithesis to the scaly habit and advanced dictyostely of *Phegopteris* and *Dryopteris*. The phyletic distinctness of the Dicksonioid and Cyatheoid series has been indicated elsewhere,<sup>1</sup> the one having constantly marginal and the other as constantly superficial sori. Thus the sources from which *Polypodium punctatum* and *Phegopteris* are derived are themselves distinct.

On similar grounds it would seem probable that *Acrophorus nodosus* finds its proper place with the Nephrodioid Ferns. Its sorus is seated sometimes on the enlarged apex of a vein, as its name implies (Fig. 41, a), but frequently it may appear laterally on a vein (Fig. 41, b); and the difference may be seen illustrated by sori on the same leaf. The indusium, which corresponds in position to that of *Nephrodium*—or indeed to that of the vestigial inner indusium of *Polypodium punctatum*—is often so small that

<sup>1</sup> Studies, III. Ann. of Bot., vol. xxvii, p. 471.

it is hidden by the sporangia when mature: but it is seen in Fig. 41, a. Thus the position and investment of the sorus are not distinctive from that seen among the Dicksonioid derivatives. But the stem and the petioles of this Fern are covered by chaffy scales resembling those of *Nephrodium*, while the Dicksonioids have hairs. The vascular system of the upright stock of *Acrophorus* is a dictyostele, and the leaf-trace is highly divided, as in *Nephrodium*. These features support the position of *Acrophorus* among the Aspidiinae assigned to it, together with *Cystopteris*, by Prantl.<sup>1</sup> Notwithstanding such soral similarities as they may present to *Hypolepis* and other Dicksonioid derivatives, these genera are to be ranked as of Nephrodioid affinity.<sup>2</sup>

I am indebted for material of *Acrophorus* to the Director of the Calcutta Garden, through Mr. Cave of Darjeeling.

#### *Monachosorum*, Kze.

Another parallel but distinct case is the Fern first described by Kunze as *Monachosorum davallioides*, and now named *M. subdigitatum* (Bl.), Kuhn. It has been ranked by various authors as *Aspidium*, *Polypodium*, *Gymnogramme*, *Phegopteris*, *Anogramme*, and *Desmopodium*. This shows the uncertainty there has been as to its position. Finally, it is retained as the single species of the genus as originally named.

Kuhn<sup>3</sup> ranked this genus as a special division (C) of his Gymnogrammeae. He relates how Kunze placed it close to *Polypodium*; Féé ranked it doubtfully with *Anogramme*; Moore, as a doubtful genus before *Polypodium*, from which genus it is sharply distinguished by habit. This would relate it best with *Leucostegia*, as Beddome has placed it. Finally, Kuhn retains it, partly on ground of habit, partly of its simple hairs, and partly of the absence of swelling of the nerve-endings, as a substantive genus; Christ ranks it with *Gymnogramme* (l. c., p. 76); Diels<sup>4</sup> places it as a substantive genus next to *Dennstaedtia*, with the remark that its habit recalls *Davallia*, but that the views of authors diverge as to its systematic position. It will be seen that the relation to *Dennstaedtia* is probably the nearest.

Material, both dried and wet, of *Monachosorum* was obtained for me by Mr. Cave from Darjeeling, through the kindness of the Director of the Calcutta Garden. It showed the external characters already described for the species, and in addition one which, though noted by Sir Wm. Hooker, seems to have passed out of sight, viz. the existence of bulbils in the axils of the primary pinnae of certain specimens.<sup>5</sup> The fact that these are present in *Monachosorum* provides a useful point of comparison with *Dennstaedtia*;

<sup>1</sup> l. c., p. 16.

<sup>2</sup> Compare Studies, II, Ann. of Bot., vol. xxvi, pp. 302-5, and especially C. Christensen, A Monograph of the Genus *Dryopteris*, 1913, p. 64.

<sup>3</sup> Die Gruppe der Chaetopterides, 1882, p. 344.

<sup>4</sup> E. and F., i. 4, p. 218.

<sup>5</sup> See Sp. Fil., vol. iv, p. 256.

for in specimens of *Dennstaedtia dissecta* (Sw.), Moore, taken in Jamaica, bulbils were found in a similar position. Their presence had already been noted by Jenman in *D. rubiginosa*, especially in plants from the higher elevations ('Synoptical List of Jamaican Ferns'). An examination of the further details in *Monachosorum* may then start on a working hypothesis of its relation to the Dennstaedtiinae, and especially to *Dennstaedtia* and *Microlepia*.

The relatively thin ascending axis bears laxly crowded leaves, and axis and leaves bear simple ferruginous hairs, not scales. This accords with the Dennstaedtiinae, and marks it off from *Davallia* on the one hand, and from *Polypodium* on the other. Transverse section of the axis shows a dictyostele

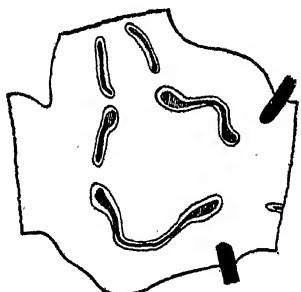


FIG. 42. Transverse section of the stock of *Monachosorum subdigitatum*. ( $\times 4.$ )

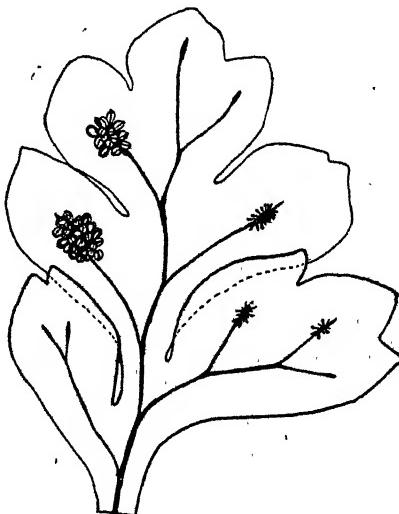


FIG. 43. Pinnule of *Monachosorum subdigitatum* in surface view. ( $\times 10.$ )

but very slightly removed from a solenostelic state (Fig. 42). The leaf-trace comes off as a very broad but thin strand, leaving a leaf-gap which very soon closes. The mode of its origin, by dilation and thinning of a region of the solenostele, closely resembles what is seen in *Denn. apiifolia*, *adiantoides*, or *cicutaria*, as shown in Gwynne-Vaughan's preparations. As the leaves are closely aggregated on the oblique rhizome, the leaf-gaps overlap in *Monachosorum*, two or three being present in a single transverse section. Such a dictyostelic state would be the natural result of the shortening of the internodes in an obliquely ascending stock like that of *Monachosorum*, starting from an elongated solenostelic stock such as that of the simpler Dennstaedtiinae. Before the leaf-trace actually separates it may have already parted in its median plane to form two isolated straps. But this is not constant. Sometimes the transverse section of the leaf-trace may be already divided into two

before the two lateral straps have themselves disengaged from the axial stele. As they pass up into the petiole they maintain their state of two equal straps. Such division of the leaf-trace occurs occasionally among the Pterids, as in *Lonchitis* and *Pteris cretica*; and it is common among the Gymnogramminae. It is not a very trustworthy basis for exact comparison. There is much sclerenchyma in isolated gritty bands, which makes the cutting of sections from mature stems difficult. This again is an unreliable character for comparison.

The sorus has been depicted by Christ.<sup>1</sup> It is without indusium, and is borne at or very near to the slightly enlarged end of a vein (Fig. 43). The sporangia are almost simultaneous in origin, and the annulus interrupted at the stalk. The spores are tetrahedral. These characters, together with habit, anatomy, and hairs, justify the position assigned by Diels in proximity to *Dennstaedtia*. It is probably a derivative along a line parallel to that of *Hypolepis* and *P. punctatum*.

#### PHYLETIC CONCLUSIONS.

All of the Ferns considered in this memoir have marginal sori, either actually so or indirectly by descent. Comparison shows that they illustrate in various degrees and in divers ways a tendency of the sorus to shift from the actual margin to a superficial position. The most gradual steps in this change of position can be traced in nearly related forms amongst them. The biological advantage gained by it in the protection of the sorus while young needs no insistence.

The ultimate origin of them all has probably been from some source such as is represented by the Schizaeaceae of the present day. *Lygodium* already shows protective growths covering its isolated marginal sporangia, which clearly prefigure the double indusium. If we imagine in each indusial pocket of *Lygodium* a plurality of marginal sporangia, the result will be very like the first state of the sorus of *Thyrsopteris* or of *Dicksonia*.<sup>2</sup> If a graduate sequence of sporangia were produced upon the same receptacle a condition would be attained not unlike what is actually seen in later stages of the sorus of a Dicksonioid Fern. Thus the Dicksonioid sorus may be traced from a Schizaeoid source, while the sporangial structure also offers palpable analogies. The anatomy as well as the sporangia of the Dicksonioids show some advance on the Schizaeoid type as seen in *Lygodium*; but both have still dermal hairs and no scales. Thus a comparison suggests a relation, though not a near one, of the Dicksonioid Ferns to the Schizaeaceae.

A main line of descent within the Dicksonioid affinity, involving progressive modifications, is indicated by the sequence of genera *Thyrs-*

<sup>1</sup> I. c., p. 76.

<sup>2</sup> Land Flora, Figs. 329, 330.

*pteris* and *Dicksonia*, *Dennstaedtia* and *Microlepia*, *Hypolepis*, *Polypodium* (*Dryopteris*, C. Chr. ?) *punctatum*, Thunbg., and *Monachosorum*. Another sequence, probably related to *Dennstaedtia* and *Microlepia*, includes *Davallia* in the widest sense, *Deparia*, *Nephrolepis*, and perhaps also *Oleandra*. In these the sorus becomes increasingly superficial. The fundamental leaf-type of all of them was the Sphenopterid, with separate ultimate lobes, each traversed by a single vein as in *Thyrsopteris*—a type essentially similar to that of *Hymenophyllum*. Webbing is, however, not an unfrequent feature. But it does not bring with it the coalescence of the sori to form fusion-sori.. In fact these Ferns are characterized by the sori maintaining their individuality as discrete developments upon the separate endings of the veins.

These Ferns may probably be seriated from *Thyrsopteris* as a relatively primitive type ; though *Loxsomopsis* may eventually turn out to be the type of a still more simple starting-point, linking on to *Loxsoma* itself. The sequences roughly sketched above may be compared on the basis of the criteria of habit, dermal appendages, vascular construction, soral position, and indusium, the receptacle and sequence of sporangia, and the characters of the sporangia themselves. Unfortunately their gametophytes are too imperfectly known to serve as a further basis for the comparison.

In habit they are mostly rhizomatous : but *Thyrsopteris* is a low Tree-Fern. It has, however, horizontal underground runners with long internodes. The habit of *Thyrsopteris* is to that of the dendroid Dicksonias as that of *Lophosoria* is to the large Cyatheas. Both are to be interpreted as having acquired the upright habit secondarily from a rhizomatous origin, which the dendroid forms carried farther. The leaves are as a rule highly divided, though webbing is frequent, and they have an open venation in all except in *Deparia Moorei*, which is a highly derivative form.<sup>1</sup>

The dermal appendages of *Thyrsopteris*, *Dicksonia*, *Dennstaedtia*, *Microlepia*, *Hypolepis*, *Polypodium punctatum*, and *Monachosorum* are all simple hairs of a characteristic brownish colour. But in *Davallia*, *Nephrolepis*, and *Oleandra* scales are present, indicating them as relatively advanced.

The vascular construction of all is based on the solenostele, which is simple in *Cibotium*, *Dicksonia*, *Dennstaedtia*, *Microlepia*, *Hypolepis*, and *Polypodium punctatum*, and is only departed from to a slight dictyostely in *Monachosorum*. But *Thyrsopteris* and *Dennstaedtia adiantoides* and *ruginosa* have in their larger axes medullary complications. The leaf-trace is as a rule undivided. All these are then relatively primitive. On the other hand, *Davallia*, *Nephrolepis*, and *Oleandra* have advanced dictyostely, with highly divided leaf-trace: characters which again indicate for them an advanced organization.

In soral position, that of *Thyrsopteris* is actually marginal, and remains

<sup>1</sup> See Thompson, Trans. R. S., Edin.

so to maturity. But in *Cibotium* and *Dicksonia*, though it is marginal in origin, it is diverted during development to the lower surface, while the upper indusium is more massive than the lower. The inequality is more marked in *Dennstaedtia* and *Microlepia*, while in *Hypolepis* the inner indusium is absent, or represented only vestigially, as in *Polypodium punctatum*. The upper indusium, however, asserts itself, receives an extension of vascular tissue from the receptacle, and takes its place in *P. punctatum* and *Monachosorum* as an apparent lobe of the lamina. The sorus itself then appears to be superficial; but comparison shows that phyletically it was marginal, and that the upper indusium, which now appears marginal, arose originally from the upper surface. Accordingly those last named are held as derivative forms. This point comes out still more clearly for *Davallia*, *Nephrolepis*, and probably also for *Oleandra*, which also will be in this respect far advanced from the original type.<sup>1</sup>

The *receptacle*, typically marginal in origin, is conical in the relatively primitive forms, such as *Thyrsopteris*, *Dicksonia*, and *Cibotium*, where there is a gradate sequence of sporangia. But the gradate order is departed from occasionally in *Dennstaedtia* and *Microlepia*, while in *Hypolepis* and *P. punctatum* the receptacle is flattened, and the succession of sporangia is definitely 'mixed'.

The same is the case in even more prominent degree in the Davalliod series, where the mixed sorus is definitely established.

The sporangium, large and short-stalked in *Thyrsopteris*, with oblique annulus and lateral dehiscence, stamps the fundamental type of the series. But *Cibotium* departs from it at once by lengthening the stalk,<sup>2</sup> while in *Dennstaedtia* and *Microlepia* this becomes more marked, and the annulus is almost completely interrupted at its insertion.<sup>3</sup> In *Davallia* this is fully carried out, and the stalk reduced in thickness and greatly lengthened.

The use of the various criteria thus followed out supports the progression suggested. The Dicksonioideae are undoubtedly a natural sequence, in which the progressive steps can be traced so as to end in types with naked superficial sori, as in *P. punctatum* and *Monachosorum*, or indusiate superficial sori, as in *Nephrolepis*. In both cases the sori are of the 'mixed' type with small long-stalked sporangia. But throughout the Dicksonioid series the individuality of the sorus is maintained.

The derivative line of the *Pteris* series is believed to be related to this series, but collateral with it. It is based upon a natural consequence of the 'webbing' of the leaf, i.e. the lateral fusion of the originally separate Sphenopterid segments to form a flattened leaf-surface, traversed by numerous veins. In this condition the sori, still marginal in origin as in the

<sup>1</sup> See Studies, III. Ann. of Bot., vol. xxvii, pp. 461-3, where developmental evidence is given.

<sup>2</sup> Land Flora, Fig. 330.

<sup>3</sup> I. c., Fig. 334 bis.

Dicksonioid Ferns, and borne on the distal ends of the veins, would form a peripheral series nearly related to one another. *The fusion in the Pteris series does not stop at the 'webbing' of the ultimate segments, but extends in varying degree to the sori themselves, which become linked laterally into fusion-sori.* A certain step towards this fusion has already been described by Goebel<sup>1</sup> in the case of *Saccoloma elegans*, which is probably related to *Microlepia*. In this case it is only the upper indusium which fuses, the lower indusium and the receptacle remaining separate. But in *Lindsaya* both the indusial lips are confluent, while the receptacles may show varying degrees of coalescence.<sup>2</sup>

The genera included in the *Pteris* series as thus characterized are *Saccoloma*, *Lindsaya*, *Dictyoxiphium*, *Paesia*, *Pteridium*, *Lonchitis*, *Histiopteris*, *Pteris* (excluding *Doryopteris*), and *Anopteris*, with *Acrostichum*, L., as an ultimate derivative. They may now be compared according to the criteria already used for the Dicksonioid series. The large majority are rhizomatous, though some, such as *Saccoloma*, *Dictyoxiphium*, *Lonchitis*, some species of *Pteris*, and *Acrostichum*, are upright; but none develop an advanced dendroid habit, and the creeping axis is prevalent for the group. The more advanced webbing of the leaves leads to larger continuous surfaces of the lamina, with entire margins, as against the more deeply cut leaves of the Dicksonioids. This is less marked in *Paesia* and *Pteridium*, but becomes a more prominent feature in such Ferns as *Histiopteris incisa*, *Lonchitis aurita*, the *Litobrochia* section of *Pteris*, and finally in *Acrostichum aureum*. In all of these Ferns the further step to reticulate venation has been made, which is the physiological corollary upon advanced 'webbing'.

Most of these Ferns have also advanced to the condition of bearing scales. These are sometimes restricted to the stock, and commonly they are associated with simple hairs. But the genera *Paesia* and *Pteridium* are distinguished by bearing hairs only, as in the case of *Dicksonia*, *Dennstaedtia*, and *Microlepia*. This indicates for them a special position.

All of the creeping types, excepting *Lindsaya*, are solenostelic, but in those in which the stem is ascending or upright a dictyostelic condition may be approached. Accessory medullary developments may appear, which in *Saccoloma*, *Pteris podophylla*, and *Acrostichum aureum* reach a high degree of polycycly, while *Pteris elata* and *Pteridium* show this in less degree. These may be held as isolated signs of structural advance, seen in the later ontogeny of certain types. They indicate a capacity in these which does not materialize, if indeed it exists, in the rest. Being so sporadic as they are, the facts cannot serve as a basis for consecutive phyletic argument.

The mature stelar state of *Lindsaya* is well known, as standing between protostely and solenostely. In this *Lindsaya* is peculiar among

<sup>1</sup> Flora, Bd. cv, 1912, p. 46.

<sup>2</sup> Studies, III. Ann. of Bot., vol. xxvii, Pl. XXXIV, Figs. 20, 21.

Ferns, taking in this respect a lower place than any of the Dicksonioid series. It is difficult to estimate the phyletic bearing of this fact. It may be taken as indicating that there is a certain degree of freedom in respect of vascular construction, and that its features do not necessarily march parallel with other characters. It is, however, a fact of interest that the *Lindsaya* condition is a phase passed through in the ontogeny of allied Ferns. This has been seen with special clearness in the case of *Histiopteris incisa*, where it may extend for a length of three internodes (Fig. 23, pp. 30, 31). *Lindsaya* may then be held to have stood still anatomically at an early stage of its ontogeny, a fact possibly related to its often climbing habit and restricted leaf-development, while others with more ample leaves progressed to solenostely and other complications.

Most of the *Pteris* series are remarkable for the persistence with which they retain in the axis the continuity of their vascular tracts. But in some cases perforation becomes a feature. It has been noted by Gwynne-Vaughan in *Dennstaedtia*. It occurs occasionally in *Lonchitis*; but it becomes a marked feature in *Pteridium*. In the leaf such interruptions of continuity claim attention. The leaf-trace of the simpler examples is undivided (*Lindsaya*, *Paesia*), and it is the same for some of the largest (*P. podophylla*). But in *Lonchitis*, *P. cretica*, &c., it comes off at once in the form of two equal straps, by reason of a median perforation. A more prominent case of this initial perforation of the leaf-trace is seen in *Saccoloma elegans* (Fig. 16); the disintegration occurs here at a lower level than in *Cibotium*.<sup>1</sup> A similar condition is seen in *Acrostichum aureum*, while in *Pteridium* also the trace comes off in several separate straps. Thus it is seen that 'perforation' either of the stele or of the leaf-trace may occur sporadically in this series. In this, as in the medullary developments, there is a recurrence of features already present among the Dicksonioids.

The type of sorus in all the *Pteris* series is referable in origin to the isolated, marginal, gradate sorus of the Dicksonioids, with its basal indusium more or less definitely two-lipped. The nearest approach to this is seen in *Saccoloma elegans*,<sup>2</sup> where the sori are still discrete on the vein-endings, the only step towards fusion being in the upper indusium. It has been shown<sup>3</sup> to be marginal in origin. It is but a slight step from this state to what is seen in *Lindsaya*,<sup>4</sup> where the fusion of both indusia and of the receptacle is often complete, while the peripheral vascular commissure links the successive receptacles into a continuous fusion-sorus. But various degrees of this fusion may be seen in individual cases, the study of which indicates how the fusion-sorus has arisen. The condition of *Paesia* and of *Pteridium* illustrates

<sup>1</sup> Land Flora, Fig. 331.

<sup>2</sup> Goebel, Flora, vol. cv, 1912, p. 47, Fig. 10.

<sup>3</sup> Studies, III. Ann. of Bot., vol. xxvii, Pl. XXXIII, Fig. 16.

<sup>4</sup> l.c., Pl. XXXIV, Figs. 20, 21.

a similar state. In the former the incompleteness of the marginal fusion-sorus is to be related to the deep cutting of the leaf. In *Pteridium*, where the fertile leaf is less deeply cut, the fusion-sorus is more continuous, and that is the condition permanently stamped on the large genus *Pteris*. Since in *Paesia* and *Pteridium* the formation of sporangia extends over the whole length of the commissure, a continuous fusion-receptacle has been formed between the indusial lips, and the lateral fusion of the sori is thus complete.

Gradual steps are shown within this series illustrating the phyletic slide of the sorus from the marginal position, which comparison with the Schizaeoids and Dicksonioids indicates as primitive, to the lower surface. It is accompanied by degradation of the inner (abaxial) indusium. The marginal initiation of the receptacle together with the superficial origin of the double indusium has been demonstrated in *Saccoloma*, *Lindsaya*, *Paesia*, and *Pteridium*, and it is seen to correspond to that in the earlier Dicksonioids; there is, however, a strong curvature during development towards the lower surface, and a pronounced inequality of the indusia, the lower being the weaker (Figs. 19, 22). It is represented in some forms merely by vestigial hairs (*Lonchitis aurita*), and is entirely absent in others (*L. hirsuta*, *Histiopteris incisa*, and the whole genus *Pteris*). With this is coupled the passage of the receptacle from the margin, as defined by segmentation. An intermediate state is seen in *H. incisa* (Fig. 24), where it is difficult in individual cases to say whether the margin does or does not give rise to the receptacle. But in *Pteris* (Figs. 29–33) it is clear that the margin grows directly on to form the upper indusium, while the first sporangia appear superficially. Such examples in related types indicate a transition of the receptacle from its primitive position on the margin to a superficial position.

The conclusion with regard to the inner indusium is important. When the whole body of comparative evidence is considered, and especially the habit-similarity and anatomy of such a sequence as *Paesia*, *Pteridium*, and *Histiopteris incisa*, the thesis appears to be well founded, *that the inner indusium has become abortive*. Biologically this may be held as a natural consequence of the downward curving of the fusion-sorus. The protective function of the lower indusium is thus taken over—as in the more advanced Dicksonioids—by the lower leaf-surface, with which the downward curved sorus is in juxtaposition. Morphologically all that remains is in some forms perhaps a few hairs, in others no vestige.

But this conclusion is the direct converse of that of Mettenius.<sup>1</sup> He developed a theory, and supported it by drawings from various closely-related species, that the inner indusium was an upgrade structure formed by the webbing together of hairs in close juxtaposition. He

<sup>1</sup> Farngattungen, iii, ‘Ueber die mit einem Schleier versehenen Arten von *Pteris*’. Frankfurt, 1858.

specially quotes *Anopteris heterophylla* as illustrating this.<sup>1</sup> The conclusion stated above takes the opposite view, that the inner indusium is a down-grade structure in these Ferns, and in course of elimination for reasons easily understood. This seems to be the only interpretation of the facts drawn from a comparative study of closely-related Ferns, provided that it is extended to a consideration of soral characters wider in its scope than the genus *Pteris* itself.

The sequence of sporangia has been found in *Saccoloma* to be graduate.<sup>2</sup> The same is seen for young stages in *Lindsaya* (Fig. 13), but later the 'mixed' state supervenes. It is clearly seen also in some cases in *Pteridium*, but this order is not maintained, and the mixed condition becomes a regular feature in matured specimens of *Pteridium* (Figs. 19, 20), as it is also from the first in *Pteris* (Figs. 31, 32). The conclusion follows that *Saccoloma* and *Lindsaya* most closely follow the more primitive graduate type; *Pteridium* takes a middle position, and *Pteris* is the most advanced in respect of the succession of sporangia.

It has been seen that in the Dicksonioideae there is a progression in sporangial character from the short-stalked type of *Thyrsopteris* with oblique and complete annulus through the intermediate types of *Cibotium*, *Dennstaedtia*, and *Microlepia*, to the long thin-stalked type with vertical interrupted annulus of *Davallia*. The sporangia of the Pteridoideae show a similar though less complete progression. It has been shown that in *Lindsaya* the sporangium is rather long-stalked, with a complete annulus.<sup>3</sup> The same is the case, though less perfectly, in *Odontosoria*.<sup>4</sup> But in *Pteridium* and *Pteris* the sporangium is of the ordinary Polypodioid type—long-stalked, and with interrupted, vertical annulus. This indicates that *Lindsaya* is in this character also relatively primitive, corresponding most nearly with the state seen in *Cibotium*, while *Pteridium* and *Pteris* are relatively advanced.

There is nothing calling for special remark in the spore-numbers of this series. They seem to have settled down to figures between 32 and 64. The spore-form appears to be inconstant. The fact, as stated by Diels,<sup>5</sup> that they are bilateral in *Histiopteris* and *Paesia*, but tetrahedral in *Pteridium*, *Anopteris*, *Pteris*, and *Monachosorum*, shows that in this affinity spore-form is not a dependable character for comparison. The character of the spore-coats, however, brought into prominence by Hannig,<sup>6</sup> presents some points of interest. Obviously the presence or absence of a perispore is not a dependable character for comparison in all cases. It is shown by Hannig's own table<sup>7</sup> that it cuts athwart the very natural group of the

<sup>1</sup> l. c., Fig. 17.

<sup>2</sup> l. c., p. 458.

<sup>3</sup> Studies, III. Ann. of Bot., vol. xxvii, Pl. XXXIV, Fig. 22.

<sup>4</sup> l. c., Fig. 19.

<sup>5</sup> l. c., pp. 287-97.

<sup>6</sup> Flora, 1911, p. 321.

<sup>7</sup> l. c., p. 339.

Blechnae, and is a feature of relatively recent types of Ferns. It must accordingly be used with discrimination. But his table certainly demonstrates its constancy in several large series, and in none better than in the Pteroideae. It is accordingly of some interest to note that *Acrostichum (Chrysodium) aureum* and *praestantissimum* agree in this respect with the Pterid series, in the absence of a perispore, therein differing from all other Acrostichoid Ferns examined. This fact detaches them from the latter, and indicates relationship to the Pterid series.<sup>1</sup>

It is thus seen that by comparison in respect of several distinct criteria drawn from the sporophyte generation, the Dicksonioids may be seriated *inter se*, and related with the Schizaeaceae, as suggesting with some degree of probability their phyletic source. The main sequence of the Dicksonioideae is based upon the maintenance of the individuality of the sorus. It is indicated roughly by the genera named in order thus:—*Thyrsopteris*, *Cibotium*, *Dicksonia*, *Dennstaedtia*, *Microlepia*, *Davallia*, *Nephrolepis*, *Oleandra*. A second series of Dennstaedtiinae, springing from *Dennstaedtia* or *Microlepia*, leads to *Hypolepis* and *Polypodium punctatum*, and related to these is also *Monachosorum*:

Another line is the Pterid series, based upon the formation of fusionsori; it is probably related in origin through the lower Dicksonioids to some Schizaeoid source. There seems to be a probability of the attachment of *Saccoloma* to *Microlepia*. The relation of *Lindsaya* is more problematical, but that genus occupies some place relatively low in the scale of the Pterid series. The main series of the latter consists of *Paesia*, *Pteridium*, *Lonchitis*, *Histiopteris*, *Anopteris*, and *Pteris*; it also includes *Acrostichum*, L. It has clearly sprung from Ferns with a Dicksonioid, two-lipped, marginal sorus, by their lateral fusion and passage to a superficial origin, with abortion of the inner indusium. Thus the Dicksonioids lead to an ultimate Polypodioid sorus, and the Pteroids to an Acrostichoid state. And both in course of their elaboration have been seen to lose their inner indusium by abortion, consequent upon the phyletic slide of the sorus to the lower surface of the leaf.

Comparison thus shows that a sorus with a single indusium may originate along more than one line from a bi-indusial source. The question will be whether all Ferns with a sorus near to the margin, and protected by a single indusium, are to be credited with a similar phyletic history. The practice of systematists has hitherto been to group all such together. It is not explicitly stated that this grouping implies their phyletic unity, but at least it may be held as indicating a view of substantial alliance.

The consequence has been that under the heading of the Pterideae there have been placed as subdivisions, in addition to the Pteridinae, the

<sup>1</sup> Compare Frau Schumann, Flora, 1915, p. 208.

Gymnogramminae, Cheilanthinae, and Adiantinae.<sup>1</sup> But it must be asked, Are all of these Ferns to be traced phyletically to a two-lipped sorus, as in the case of the Pteridinae, or as I prefer to call them, the Pterid series? It is only on the basis of detailed examination and comparison that this question can be resolved. But such examination must be held over for the present, with the simple remark that there is no apparent reason for holding as justifiable the assumption that they have been so derived.

#### SUMMARY.

1. The stelar ontogeny of *Schizaea* and *Anemia* starts from protostely, a condition retained in the adult *Lygodium*. It then progresses to the medullated monostele, a state retained by the adult in *S. pusilla* and *rupestris*, and apparently also in *S. fistulosa*. In *S. dichotoma*, and less perfectly in *S. malaccana*, it progresses further to 'ectophloic siphonostely'. In *Anemia* (*§Anemiorrhiza*) the development proceeds to 'amphiphloic siphonostely' (= solenostely), and in *Eu-anemia* and *Mohria* to dictyostely. These states may be held as indicating successive steps of stelar advance, the ontogeny being less completely carried out in some forms than in others.

2. In *Schizaea rupestris* and *digitata* the sporangia have been shown to be of marginal origin, and the later-formed indusium originates superficially below the sporangia. This condition prefigures that seen in other Marginales.

3. Within the Pterideae of Prantl there are two probably distinct lines of phylesis. The first may be styled 'Pterideae bi-indusiatae', or better, the Pterid series; the second the 'Pterideae uni-indusiatae', or better, the Cheilanthoid series. The latter will be held over for the present. The former, which include *Lindsaya*, *Paesia*, *Pteridium*, *Lonchitis*, *Histiopteris*, *Anopteris*, *Pteris*, and *Acrostichum*, L., are traceable from a two-lipped Dicksonioid origin. Perhaps *Saccoloma* may also be included.

4. The *Lindsaya*-type of stele has been verified for eighteen species of the genus, and may be accepted as a good generic character. The genus is further characterized by its 'fusion-sorus', with vascular commissures.

5. In its ontogeny the fusion-sorus of *Lindsaya* is actually marginal, the indusial flaps originating superficially below it. The sporangia show at first a gradate sequence, but soon younger sporangia are interpolated, and a mixed condition is assumed.

6. In *Dictyoxiphium* the fusion-sorus differs from that of *Lindsaya* chiefly in the absence of the upper (adaxial) indusium.

7. Except for amendments of detail the stelar system of *Saccoloma* has been shown to accord with Mettenius's description.

8. The fusion-sorus of *Pteridium* is marginal in origin, with two

<sup>1</sup> Compare Diels, Natiirl. Pflanzenfam., i, 4, p. 254.

indusial flaps, as in *Lindsaya*. It shows a graduate sequence of sporangia, passing soon to a mixed condition.

9. *Paesia viscosa*, like *P. scaberrula*, has a typical solenostele. Its marginal sorus is usually two-lipped, but shows inconstancy of the inner (abaxial) indusium.

10. *Lonchitis* shows in vascular characters, in investiture, in venation, and in soral features an intermediate position between the bi-indusiate types and the genus *Pteris*.

11. *Pteris (Histiopteris) incisa* closely resembles *Pteridium* in habit; it is advanced as regards scales and venation, but it is less complex in stelar condition. Its fusion-sorus shows fluctuations from the exact marginal origin, while the inner indusium is absent. After signs of basipetal sequence of sporangia the sorus passes over to the mixed condition.

12. The genus *Pteris* (excluding *Doryopteris*) has superficial scales as well as hairs. Its stelar structure is either solenostelic or some near derivative, sometimes with a high degree of elaboration (*P. podophylla*). The leaf-trace may be a single or double strap. The venation is frequently reticulate. The fusion-sorus is superficial in origin, and the inner (abaxial) indusium is absent. The succession of sporangia is mixed. All these characters indicate advance upon the *Lindsaya-Paesia* type.

13. No support is found for Mettenius's theory of the origin of the inner indusium by fusion (*connatus*) of hairs. On the other hand, it appears that isolated hairs may in some cases replace a decadent inner indusium.

14. *Acrostichum praestantissimum* and *A. aureum* appear to be 'Acrostichoid' derivatives of *Pteris*, sprung from some *Litobrochia*-type, such as *P. (L.) splendens*.

15. It has thus been shown that either the outer (adaxial) or the inner (abaxial) indusium may be abortive in the Pterid series. In the Dicksonioidae steps of abortion of the inner indusium are seen in *Dennstaedtia*, *Hypolepis*, *Polypodium punctatum*, and *Monachosorum subdigitatum*.

16. In *Hypolepis* and *Monachosorum* the outer (adaxial) indusium may receive a vascular supply from the receptacle, and appear flattened as a marginal lobe of the pinnule.

17. Phyletically *Hypolepis* and *Monachosorum* are derivatives of the Dicksonioid-Dennstaedtioid series. *Acrophorus* and *Cystopteris* are distinguished from these by their scales and advanced dictyostely, and are related to the Nephrodiod Ferns.

18. The large series of the Dicksonioids are characterized by the sori maintaining their identity as discrete developments on the separate endings of the veins. They have dermal hairs, not scales, excepting their Davalliid derivatives.

19. The Dicksonioids probably sprang ultimately from some Schizaeoid

source, through types of the nature of *Loxsomopsis* and *Thyrsopteris*. They culminated in the Davalliod sequence.

20. The Pterid series are distinguished from the Dicksonioids by the lateral fusion of their marginal sori, which are linked together by vascular commissures.

21. They are related to the Dicksonioids as a collateral branch, attaching probably in the neighbourhood of *Microlepia*. They culminated in *Acrostichum*, L.

22. The Cheilanthroideae have usually been ranked with the Pterids. But until they have been tested by detailed examination, there is not sufficient reason to assume a near phyletic relationship.

23. All the Ferns here treated belong to the Marginales. In the methods of their advance they show analogies with the Superficiales, and in particular this is seen in those of their forms which have apparently superficial sori. These are here shown to result from a slide of the marginal sorus to a superficial position. In some cases this is ontogenetic, in others phylogenetic as revealed by comparison.

24. The Superficiales are believed to represent Ferns in which that slide took place so early in their descent that the two sequences must be held to be phyletically distinct, notwithstanding those analogies.

# Studies in the Permeability of the Pulvinus of *Mimosa pudica*.

BY

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. AND .

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With five Figures in the Text.

AS a result of the work of numerous investigators (see the summaries by Pfeffer (1) and Jost (2)), it may be considered as established that the movement of the pulvinus of *Mimosa pudica* under the stimulus of shock (the so-called seismonastic movement) is due to a change in the turgor of the cells. The change in the shape of the pulvinus is mainly brought about by a loss of turgor in the cells of the lower half of the pulvinus; the loss of turgor allowing of the elastic recoil of the stretched cell wall, assisted under normal conditions by the weight of the leaf and the tension of the upper half of the pulvinus. The loss of turgor appears to be associated, as is to be expected, with a loss of water from the cell.

But although the seismonastic movement of *Mimosa* is associated with a turgor change yet the mechanism of that change remains uncertain. Pfeffer has pointed out that the reduction of turgor is most likely to be due either to a reduction in the quantity of osmotically active substances in the cell, thus allowing water to escape from the cell, or to an increase in the permeability of the cell, thus allowing of the escape of some of these osmotic substances together with some of the water of the cell-sap. The second hypothesis, that of the increase of protoplasmic permeability to such a degree as to bring about a serious reduction in the cell turgor, is difficult of acceptance as a working hypothesis since there is no obvious mechanism by which the osmotic substances which thus escape in solution can be drawn back so as to allow the cell to regain its turgor.

Lepeschkin (3) has shown that the pulvinus of *Mimosa* and of *Phaseolus* is sensitive to light, the permeability of the cells being increased when illuminated; he associates the nyctinastic movements of the leaves

with these changes in permeability. Owing perhaps to the observations and conclusions of Lepeschkin with reference to nyctinastic movements the view seems to have gained ground, in spite of the inherent objection already cited, that a similar change of permeability will explain the response of *Mimosa* to shock.

It seemed of importance that this view should, if possible, be subjected to the test of experiment. A method to be suitable must be one by which the exosmosis of very small quantities of the cell-contents can be recognized, for it would be difficult to work with more than a single pulvinus. As the increase of permeability, if it occurs, would almost certainly lead to the increase of rate of exosmosis of *electrolytes* as well as of *non-electrolytes*, it seemed likely that the electrical conductivity method for the estimation of dilute solution of electrolytes could be used to solve this question.

Experiments using this method were begun some years ago; since that time the method has been used in many investigations on cell permeability. The erratic behaviour of the pulvini, i. e. the refusal of many of them to respond, after removal from the plant, to shock stimuli, has delayed the work.

#### EXPERIMENTAL.

The method employed was essentially the same throughout; a pulvinus of *Mimosa pudica* was removed from the plant and immersed in a small volume of 'conductivity water' distilled over glass. The rate of exosmosis of electrolytes was determined periodically by the electrical conductivity method.

After removal of the leaf from the plant by a clean cut at the base of the pulvinus the upper part of the petiole was cut away so that a length of about an inch and a half remained attached to the pulvinus. In order to increase the rate of diffusion the internal tissue was further exposed by slicing away an equal portion of tissue from each side of the pulvinus, but leaving the upper and lower surfaces in the main intact. This operation was made with a sharp scalpel and as speedily as possible. The pulvinus was immediately placed in water so as to wash away the contents of the cut cells before beginning the experiment. The petiole was then gripped by a split rubber cork held in a cork-borer, which was lowered by a rack and pinion until the pulvinus was completely immersed in the liquid of the conductivity 'cell'. Treatment of the pulvinus in this way did not destroy its power to contract when stimulated. In a great many cases response to stimulation was obtained within fifteen minutes from the time of removal of the pulvinus from the plant; in others a period of some thirty or thirty-five minutes elapsed before the pulvini becomes stimulable. The unstimulable condition seemed to be associated with extreme turgor of the cells of the under side of the pulvinus, since it was frequently observed that the

contour of this side during the dormant period was convex, but when it returned to the normal, slightly concave condition the response to stimuli was again established. As already stated, for some reason unexplained some pulvini never recovered from this dormant condition. In one case, however, a pulvinus, which during two hours had given no response to stimulation, was left immersed in the cell at 4 p.m., and was found two hours later to have recovered. The factors which determine this behaviour were not discovered, and a certain element of doubt always existed as to

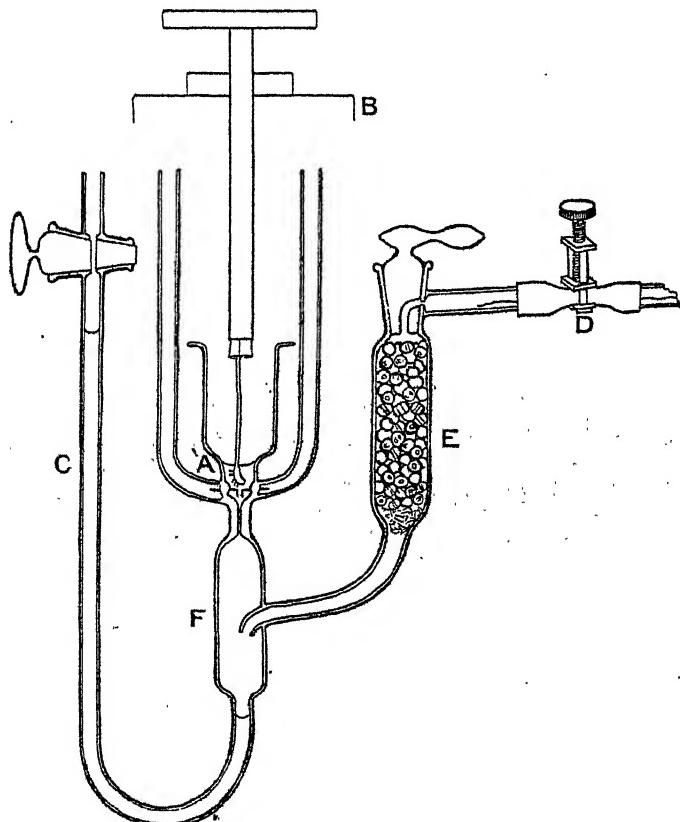


FIG. I.

whether a given pulvinus would behave normally. Such cases of failure to obtain response to stimulation were in the later part of the work reduced to one in ten.

The conductivity cell employed was designed for the purpose, and is specially serviceable for conductivity determinations where the volume of liquid available is very small. The actual cell A (Fig. 1) was a small glass cup six or seven millimetres in diameter with electrodes, two millimetres apart, fused in at the sides; the electrodes had an area of about two square

millimetres each and were made from platinum wire hammered flat. In order to increase their surface the electrodes were platinized and roasted before insertion. The capacity of the cell was about 1 c.c. and the volume of liquid usually employed was between 0.5 c.c. and 0.7 c.c. The 'cell constant', which naturally varied with the height to which the cell was filled, was of the order of 3 to 4. Above the cell the apparatus widened out to a cup-shaped mouth which could be closed with a cork when not in use. During an experiment this mouth was left open to air, but the liquid in the cell was protected from dust falling vertically by a wide tin lid B, perforated in the centre and soldered to the cork-borer which carried the pulvinus.

The liquid was agitated by a regular intermittent stream of air-bubbles, a system which proved very efficient and at the same time prevented the accumulation in the liquid of carbon dioxide produced by the respiration of the pulvinus. The air was forced from a gas-holder and entered at the base of the cell through a capillary of such narrow dimensions that a considerable pressure was required. The magnitude of this pressure was observed in the open side tube C shown on the left in the figure. The pressure alternately rose to about 13 cm. of water and fell to about 12 cm. At the maximum pressure air entered the cell in a rapid stream of some five or six bubbles. A short interval of time then elapsed before the maximum pressure was again attained; this interval could be regulated by means of the screw-clip D compressing the rubber tubing upon a wire passing through it. In most of the experiments this interval was approximately ten seconds. The whole apparatus was placed in water in a thermostat with glass sides. The air before entering the cell was saturated with water vapour at the temperature of the thermostat by passage over moist glass beads in the tube E immersed in the bath. In this way evaporation of the liquid in the cell was reduced to a minimum. In order to prevent electrolytes being carried into the cell the splash-trap F was interposed between this vessel E and the capillary entrance to the cell. Most of the experiments were carried out at temperatures between 25° and 40° C.

For estimations of conductivity, instead of the usual method of Kohlrausch, an ordinary moving coil galvanometer and a rotating commutator of the Fitzpatrick (4) type was employed. Owing to the weakness of the solutions and the high constant of the cell, a high balancing resistance had to be used; one variable between 10,000 and 100,000 ohms, made by Gambrell Bros., was usually employed.<sup>1</sup> In all cases the rate of increase of conductivity was taken as the measure of the rate of exosmosis.

<sup>1</sup> In order to make certain that the accuracy of the readings was not vitiated by the capacity of the resistance, which was of the ordinary type with non-inductive winding, comparative experiments were made with a resistance of the same range made of 'anti-capacity gauze' by W. Paul. The results were found to be identical.

Various methods were used for stimulating the pulvinus, such as mechanical shock by touching or cutting the top of the petiole, electrical stimulation with the help of an induction coil, and stimulation by sudden alteration of temperature. The last method was found to be most convenient, for the stimulus could be applied by simply raising the pulvinus out of the warm fluid. The sudden transition to the cooler air above the fluid acts as a stimulus and brings about contraction.

An experiment was conducted in the following way :

The condition of the cell was tested by filling it with conductivity water which was kept continually stirred by means of a slow stream of bubbles. The conductivity was then noted periodically, and the cell was considered to be satisfactory when the rise of conductivity was not above a certain rate ; owing to the cell being made of ordinary glass and to very slight evaporation from the fluid there was always a steady, slight rise of conductivity. This 'washing factor' could be allowed for, if necessary, in the later readings. The cut pulvinus, previously washed, was then inserted. The rate of exosmosis from the cells indicated by the rate of increase of conductivity was then determined periodically, the fluid being changed, if necessary, after the first few minutes. Stimulation was brought about by raising, by means of a rack-and-pinion adjustment, the cork through which the petiole was threaded, care being taken that the pulvinus did not come in contact with the sides of the cell. If the petiole was raised only sufficiently to bring the pulvinus just above the fluid no stimulation resulted, but if the pulvinus was raised 25 to 30 mm. above, so that the fall in temperature was considerable, stimulation was quickly effected in favourable cases, and the end of the pulvinus showed the characteristic curvature after the lapse of a few seconds. After a period of time (often 30 sec.) which was constant for each experiment, the pulvinus was returned to the fluid. Readings were taken at regular intervals of  $\frac{1}{2}$ , 1, 2, 3, or 5 min. The bubbling was continued throughout the experiment, being stopped only for a definite period to allow of a reading being taken.

As the permeability of the pulvinus was found to be affected by light changes (see later) the experiments were usually conducted with the pulvinus brightly illuminated by means of a Nernst burner (micro-Nernst lamp of Zeiss).

TABLE I.

*Effect of Contraction on the Rate of Exosmosis from the Pulvinus of Mimosa pudica.*

August 3, 1915. Pulvinus with sides sliced. Temperature  $39.9^{\circ}\text{C}$ . Illuminated by Nernst lamp. Stimulated at intervals of fifteen minutes by raising the pulvinus into cold air for thirty seconds while taking the reading of the conductivity. At other times the readings were taken with the

pulvinus raised just above the liquid where the air was only slightly below the temperature of the bath; this, as seen below, caused no contraction of the pulvinus.  $A$  and  $A'$  give the angles which the pulvinus made with the petiole before and after raising.

<i>Time in Minutes.</i>	<i>Treatment of Pulvinus.</i>	<i>A.</i>	<i>A'.</i>	<i>Conductivity in Gemhos.</i>	<i>Rate of Exosmosis.</i>
0	Leaf cut	—	—	—	—
5	Experiment started	125°	—	—	—
10	Raised	143°	141°	56·2	—
15	,, and stimulated	157°	112°	62·4	6·2
20	,,	141°	141°	68·2	5·8
25	,,	153°	153°	72·6	4·4
30	,, and stimulated	154°	127°	76·3	3·7
35	,,	151°	154°	80·6	4·3
40	,,	154°	154°	84·1	3·5
45	,, and stimulated	154°	118°	86·8	2·7
50	,,	145°	145°	90·9	4·1
55	,,	149°	149°	93·3	2·4
60	,, and stimulated	149°	114°	95·6	2·3
65	,,	143°	143°	98·8	3·2
70	,,	143°	143°	100·9	2·1
75	,, and stimulated	143°	110°	102·7	1·8
80	,,	136°	136°	105·8	3·1
85	,,	136°	136°	107·7	1·9
90	,, and stimulated	136°	108°	109·3	1·6
95	,,	120°	122°	112·4	3·1
100	,,	135°	133°	114·0	1·6
105	,, and stimulated	134°	106°	115·5	1·5
110	,,	117°	117°	118·7	3·2
115	,,	125°	125°	120·0	1·3
120	,, and stimulated	125°	95°	121·0	1·0
125	,,	110°	110°	123·6	2·6
130	,,	110°	119°	124·9	1·3
135	Experiment ended	119°	—	125·7	0·8

The result shown in Table I is typical of many. It shows that the cut pulvinus kept immersed in warm water will exhibit a large number of contractions when suitably stimulated. The mere fact that the pulvinus will continue to respond to stimulation under these conditions is sufficient in itself to negative the hypothesis that the loss of turgor on contraction is dependent on an escape of osmotic substances from the tissues. For, with the internal tissues of the pulvinus freely exposed to warm water not only at the base but at the sides, such substances, if they left the cell, would pass rapidly into the surrounding fluid. There these substances would be lost to the tissues; for it is hardly conceivable that they could be reabsorbed, at least within any short period. Again, it is hardly possible that, if at each contraction there is a loss of osmotic substances sufficient to markedly reduce the turgor of the cells, such substances could be produced again at such a rate as to allow the pulvinus to continue to contract every fifteen minutes during two hours.

In nearly all the experiments a slow exosmosis of electrolytes from the cut surfaces of the pulvinus is to be observed. The rate of this exosmosis, apart from contractions of the pulvinus, falls off steadily for a long period.

The fall in the early period is possibly due to the washing away of substances derived from the cut cells, but it may be that there is an actual decrease of permeability as a result of the washing out of salts from the living cells as indicated by some of Loeb's experiments on eggs. The fall in the rate of exosmosis cannot be due to a fall in the diffusion gradient between the living cells and the surrounding medium. For a dead pulvinus may raise the conductivity of the fluid to 400 gemhos (which corresponds to about N/10 NaCl), and as the volume of the pulvinus is only 0.1, or less, of that of the surrounding fluid the concentration of electrolytes in the cells would seem to be of an order corresponding to several thousand gemhos.

Turning now to the actual rate of exosmosis in relation to contraction we find that the response to stimulation is associated with an increased loss by exosmosis; the increased loss being of the order of 2-3 times, as seen in the readings of the second hour. In the earlier period of the experiment the greater rapidity of the fall in what one may call the normal rate of exosmosis tends to reduce the apparent effect of contraction in increasing the rate of loss.

As, however, the rate of normal loss of electrolytes under the conditions of the experiment is so small that the pulvinus may be subjected to it for many hours without loss of power of response, it is clear that an increase of that loss during five minutes to two or three times the normal can in no way account for the loss of turgor of the cells. It is clear then that contraction of the pulvinus is not associated with an increase in the permeability of the cells to electrolytes in any way sufficient to produce such a loss of turgor of the cells as would explain the contraction.

These experimental results give no direct information as to the behaviour of non-electrolytes such as sugars, and it might be suggested that a rapid loss of these substances from the cell would explain the fall in turgor of the cells of the lower half of the pulvinus. It is, however, improbable that there should be a very marked increase of permeability in relation to non-electrolytes unaccompanied by any corresponding increase in the rate of exosmosis of electrolytes. Apart from this, however, the repeated contractions and recoveries of the cut pulvinus when immersed in warm water seem to exclude, as already pointed out, any explanation of the contraction which is based on the passage of osmotic substances out of the cells, whether those substances be electrolytes or non-electrolytes.

The increased loss of electrolytes associated with the contraction of the pulvinus may be due to a change of permeability associated with stimulation or it may be a mere secondary mechanical effect, the contraction of the pulvinus squeezing a certain amount of material out of the intercellular spaces or cell walls. An experiment with the immotile pulvinus of *Sapindus emarginatus* suggested that here also cooling affected the rate of exosmosis in spite of the absence of contraction. An experiment with a pulvinus of *Mimosa* which was not responsive to stimuli also gave the same result.

These experiments suggest that the sudden cooling in itself affects the permeability of the tissues, but the experiments were not carried far enough to enable this point to be decided. The difficulty of obtaining material of sliced pulvini which would give quantitatively comparable results was a bar to more accurate work.

TABLE II.

*Effect of Contraction on the Rate of Exosmosis from the Pulvinus  
of Mimosa pudica.*

October 14, 1915. Pulvinus with sides sliced away almost to the vascular bundle. Temperature 39.4°C. Illuminated by Nernst lamp. Stimulated by touching with platinum wire.  $A$  and  $A'$  give the angles which the pulvinus makes with the petiole before and after stimulation.

Time. Mins.	$A$ .	$A'$ .	Gemhos.	Rate of Exosmosis.	Time. Mins.	$A$ .	$A'$ .	Gemhos.	Rate of Exosmosis.
0	Leaf cut								
11	170°	—	27.8	—	31	169°	150°	Stimulated	
12	170°	—	28.7	0.9	32	155°	—	38.0	0.7
13	170°	—	29.5	0.8	33	160°	—	38.4	0.4
14	170°	—	30.2	0.7	34	164°	—	38.8	0.4
15	170°	—	30.7	0.5	35	166°	—	39.1	0.3
	170°	161°		Stimulated		167°	—	39.4	0.3
16	164°	—	31.3	0.6	36	155°	—	40.2	0.8
17	168°	—	31.9	0.6	37	160°	—	40.6	0.4
18	170°	—	32.4	0.5	38	163°	—	41.0	0.4
19	170°	—	32.8	0.4	39	165°	—	41.3	0.3
20	170°	—	33.3	0.5	40	166°	—	41.6	0.3
	170°	152°		Stimulated		166°	147°	Stimulated	
21	157°	—	33.7	0.4	41	154°	—	42.4	0.8
22	—	—	34.2	0.5	42	157°	—	42.7	0.3
23	168°	—	34.5	0.3	43	161°	—	43.1	0.4
24	170°	—	34.9	0.4	44	163°	—	43.4	0.3
25	170°	—	35.2	0.3	45	164°	—	43.6	0.2
	170°	150°		Stimulated		164°	146°	Stimulated	
26	157°	—	35.8	0.6	46	150°	—	44.3	0.7
27	163°	—	36.1	0.3	47	155°	—	44.7	0.4
28	—	—	36.7	0.6	48	150°	—	45.1	0.4
29	168°	—	37.0	0.3	49	162°	—	45.4	0.3
30	169°	—	37.3	0.3	50	163°	—	45.7	0.3

That cooling itself is not responsible for the slight increase of exosmosis of electrolytes associated with contraction is shown by the results given in Table II, where stimulation was brought about by touching the pulvinus with a loop of platinum wire which hung beside the pulvinus in the water of the conductivity cell. The pulvinus was thus kept at constant temperature; but the increase of exosmosis is well marked, especially in the second half of the table. The readings were taken every minute in this experiment, thus their accuracy is not very high. It is possible, though not probable, that part of the increase may be due to mechanical injury produced by contact of the wire with the tissues. The fact that most of the increase appears in the first minute after stimulation suggests that cell injury is not responsible

or the result. The association of increased permeability with contraction, apart from cooling or mechanical stimulation, is well shown in Table III, where the contractions are autonomous.

It is to be noted that in Tables I and II the pulvinus recovers less and less fully after each contraction, so that the angle in column *A* gets less as the experiment proceeds. On the other hand, the extent of the contractions seems to get greater and greater, so that the angles reached after contraction, and shown in column *A'*, also tend to get less. This is well shown in Fig. 2.

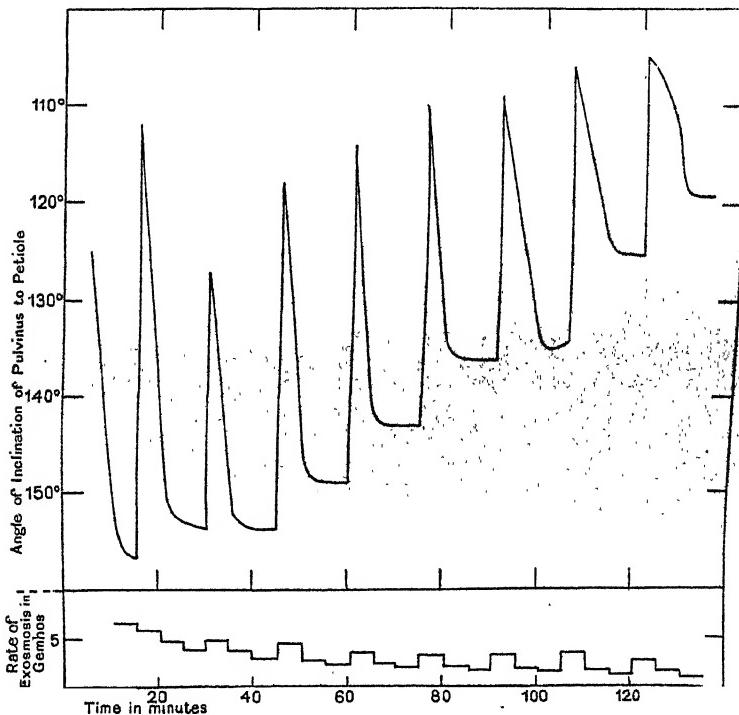


FIG. 2.

The observations here described show that the loss of turgor in the cells of the lower half of the pulvinus cannot be explained by a rapid exosmosis of osmotic substances. The loss of turgor must almost certainly be due to the disappearance or inactivation of these substances. As to the mechanism of this process we have at present no knowledge; it is suggested that changes in the adsorptive power of the colloids of the cell play a large part.

#### AUTONOMOUS CONTRACTIONS OF THE PULVINUS.

While carrying out these experiments a very interesting and, in the case of *Mimosa*, an entirely new phenomenon was met with, that of a series of rapid contractions of the pulvinus similar to those due to shock, but

autonomous or autogenic in nature, i.e. occurring when the external conditions of temperature, light, &c., were quite constant.

The first occasion on which they were observed was on April 4, 1913, when a pulvinus was placed in water in the cell at a temperature of  $29.9^{\circ}\text{C}$ . and brightly illuminated. Twenty-nine minutes after cutting it reacted to the stimulus of cooling, and after several stimulations in this way when replaced in the fluid it exhibited a series of nine contractions. The interval between two successive contractions was at first about 4 min., but it gradually decreased till the interval between the last and penultimate contractions was reduced to 1 min. 53 sec.

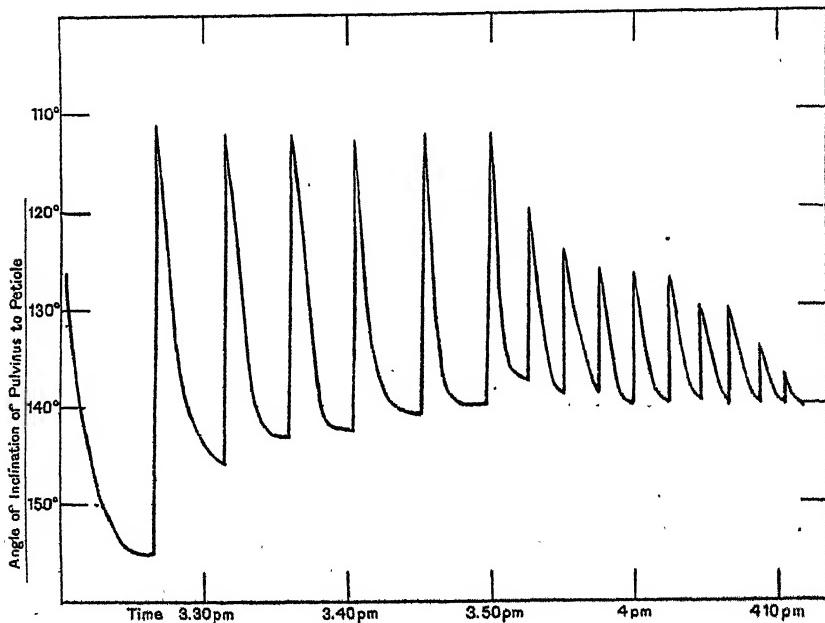


FIG. 3.

This striking phenomenon was observed on some ten occasions at temperatures between  $30^{\circ}$  and  $40^{\circ}\text{C}$ .; in all cases the pulvinus was brightly illuminated. The contractions as they proceeded were always found to occur at shorter and shorter intervals, with the result that the extent of the contractions gradually decreased, since the pulvinus had less and less time to straighten between successive contractions; the pulvinus thus finally came to rest in the partly contracted state. A graphic representation of one of these series showing sixteen autonomous movements is given in Fig. 3.

Bose (5) has observed a similar series of movements following on a single stimulation in *Biophytum* and *Averrhoa*, and he applies to them the term 'multiple response', pointing out, however, that no sharp line can be drawn between multiple responses and autonomous movements.

Bose describes in *Mimosa* a multiple *electrical* response as the result of one strong stimulation; our results, however, show that under appropriate conditions *Mimosa* may, like *Biophytum*, exhibit temporarily a series of movements which can be closely compared with those of *Desmodium gyrans*.

The high degree of sensitiveness brought about by a bright light and high temperature is no doubt related to this striking phenomenon.

Details of an experiment in which autonomous contractions appeared are given in Table III. It is to be noted that the increase in the rate of exosmosis is visible after each response in spite of the absence of either cooling or of mechanical stimulation.

TABLE III.

*Effect of Autogenic Contraction on Exosmosis from the Pulvinus of Mimosa.*

October 6, 1913. Pulvinus with sides sliced. Temp. 35.0° C. Illuminated by 'Lilliput' arc lamp focused on pulvinus by lens. Stimulation by cooling was followed by three autogenic responses during the first seventy minutes. The liquid was then changed and a sequence of eight more autogenic responses followed the initial stimulation by cooling.

Time. Min.		Gemhos.	Diff. 1 min.	Diff. 3 min.
0	Leaf cut	—	—	—
73	Pulvinus raised; the liquid changed	—	—	—
75		18.5	—	—
78		24.4	—	5.9
79.5	Autogenic response	—	—	—
81		30.1	—	5.7
84		34.2	—	4.1
85	Autogenic response	—	—	—
87		39.7	—	5.5
90		43.4	—	2.7
91	Autogenic response	—	—	—
93		46.8	—	4.4
95	Autogenic response	—	—	—
96		50.8	—	4.0
97		51.7	0.9	—
98		52.3	c.6	—
98.75	Autogenic response	—	—	—
99		53.4	1.1	—
100		54.9	3.5	—
101		55.8	0.9	—
102		56.6	0.8	—
102.75	Autogenic response	—	—	—
103		58.2	1.6	—
104		59.1	0.9	—
105		60.0	0.9	—
106		60.8	0.8	—
106.75	Autogenic response	—	—	—
107		62.3	1.5	—
108		63.3	1.0	—
109		64.0	0.7	—
110		64.6	0.6	—
110.75	Autogenic response	—	—	—
111		65.8	1.2	—
112		66.8	1.0	—
113		67.5	0.7	—

### EFFECT OF LIGHT ON PROTOPLASMIC PERMEABILITY OF THE PULVINUS.

That light increases the permeability of the protoplasm has been demonstrated by Lepeschkin (3) and Tröndle (6). The former demonstrated the effect of light on the permeability of the cells of the pulvinus of *Phaseolus* and of *Mimosa*, mainly by the use of the indirect plasmolytic method; but he also showed that the total exudate from a number of pulvini of *Mimosa* in water was greater in the light than in the dark. Tröndle relied entirely on the indirect plasmolytic method in his study of the mesophyll cells of the leaf of *Buxus* and *Tilia*.

It is clear that the conductivity method provides an almost ideal one by which the effect of light on the permeability to electrolytes can be studied, for the method can be made sensitive to extremely small quantities and readings can be for any period of time and the same organ can be used continuously throughout the investigation.

The relation of light to permeability was not studied exhaustively, but a few experiments were made to test the value of the method. The pulvinus was prepared in the ordinary way with the sides sliced to increase the surface for diffusion, and it was illuminated through the glass side of the bath by a Nernst lamp of 50 candle-power placed 10 cm. from the pulvinus. As it was considered possible that the temperature of the water in the conductivity cell might be of a higher temperature than that of the bath, owing to the greater absorption of light by the green-coloured pulvinus, some of the experiments were made with a thermo-couple in the water of the 'cell' and in the bath. A difference of not more than  $0.3^{\circ}\text{C}$ . was observed, which would mean that the readings in the light and dark would only differ by 0.6 per cent. from this cause. Owing to the magnitude of the effect of light such a difference is, of course, quite negligible.

Occasionally a pulvinus was met with which refused to react to light for unknown reasons, but the majority showed a very marked reaction, the rate of exosmosis of electrolytes, as measured by the increase of conductivity, being greatly increased. The graph shown in Fig. 4 shows the results obtained during an experiment of which Table IV gives the details. The increase in conductivity, measured in gemhos, for successive periods of 15 min. is plotted against time; the periods of darkness are indicated by the dark lines on the axis of the abscissae. It will be seen that the pulvinus was kept in darkness during the first hour; on exposure to light exosmosis increased during the first half-hour, then, although still illuminated, the permeability began to decrease; when placed in darkness for a second period an initial rise in the rate of exosmosis was observed. These effects were repeated on further changes from darkness to light and from light to darkness.

TABLE IV.

Effect of Light and Darkness on the Rate of Exosmosis from the Pulvinus of *Mimosa*.

March 24, 1913. Pulvinus slit down axis in the anterior-posterior plane, washed well with water and placed in darkness. Experiment started 12.7 p.m. Temp. 26.1° C.

Time. p.m.	Gemhos corr. to 26.4° C.	Diff. 15 min.	Time. p.m.	Gemhos corr. to 26.4° C.	Diff. 15 min.
<b>Darkness</b>					
<i>h. m.</i>					
12 10	24.8	—	3 40	156.8	2.8
12 25	44.2	19.4	3 55	161.3	4.5
12 40	54.9	10.7	4 10	165.2	3.9
12 55	63.5	8.6	4 25	167.9	2.7
1 10	70.3	6.8	4 40	170.0	2.1
<b>Daylight and Nernst lamp</b>					
<i>h. m.</i>					
1 25	81.6	11.3	4 55	173.6	3.6
1 40	96.0	14.4	5 10	176.2	2.6
1 55	108.3	12.3	5 25	178.3	2.1
2 10	119.3	11.0	5 40	179.7	1.4
<b>Darkness</b>					
<i>h. m.</i>					
2 25	131.2	11.9	5 55	181.2	1.5
2 40	140.0	8.8			
2 55	147.2	7.2			
3 10	152.2	5.0			
3 25	154.0	1.8			

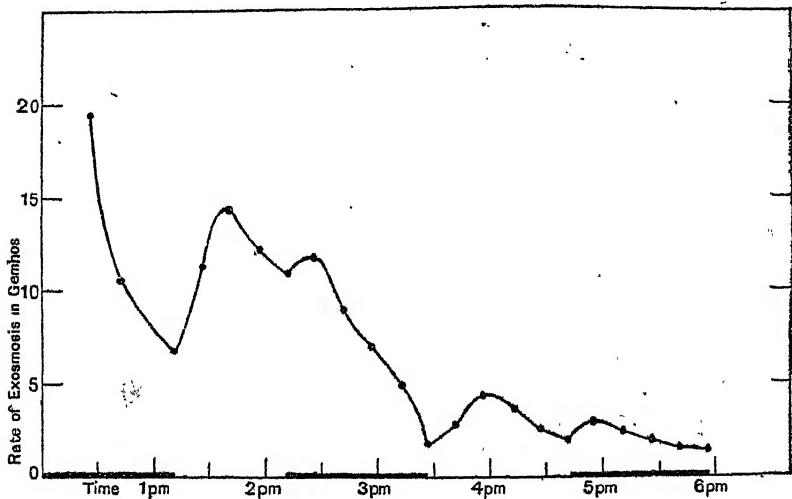


FIG. 4.

This and other experiments brought out very clearly: (1) That light has a marked effect in increasing permeability to electrolytes; (2) that the effect takes some time to reach a maximum, and then, after a varying period of time, begins to fall off; (3) that the sudden change from light to darkness also increases the permeability.

This last phenomenon is well seen in the 'humps' in the curve after the withdrawal of light. This 'after-effect' of light was shown in a number of cases.

These results fully confirm the main result obtained by Lepeschkin and Tröndle, that light increases the permeability of the protoplasm for salts. The results show also that the method here used, besides being direct, is much superior to that employed by those workers, in that it is far more delicate and is also capable of giving practically continuous records. An exhaustive study by this method of changes of permeability due to light, changes which are probably photochemical in origin, would be of great value.

#### EFFECT OF TEMPERATURE ON RATE OF EXOSMOSIS.

A few experiments were carried out by the conductivity method to investigate the effect on permeability of slow rise of temperature.

All the experiments showed that with a rise at the rate of about  $5^{\circ}\text{C}.$  in 20 min. the effect of rise of temperature on the rate of exosmosis of electrolytes was very slight up to  $52^{\circ}$ . Only when the temperature was likely to produce quickly a harmful, and no doubt irreversible, effect was there a marked increase of permeability.

TABLE V.

#### *Effect of Temperature on the Permeability of the Pulvinus of Mimosa.*

December 17, 1913. Pulvinus with sides sliced, washed in cold water for one hour and a quarter. Illuminated by daylight only. Temperature raised gradually from  $39^{\circ}\text{C}.$  to  $64^{\circ}\text{C}.$ .

Time in Minutes.	Temperature. Degrees C.	Conductivity. Gemhos.	Rate of Increase of Conductivity.
0	38.0	26.8	—
6	39.15	30.1	3.3
12	41.15	33.1	3.0
18	43.0	36.0	2.9
24	44.55	39.2	3.2
30	46.25	41.9	2.7
36	48.05	44.8	2.9
42	49.5	48.0	3.2
48	50.9	51.6	3.6
54	52.3	55.4	3.8
60	53.65	61.1	5.7
66	54.95	69.6	8.5
72	56.15	81.2	11.6
78	57.3	110.6	29.4
84	58.25	188.2	77.6
90	59.45	279.1	90.9
96	60.35	345.4	66.3
102	61.25	378.4	23.0
108	62.1	394.0	15.6
114	62.9	401.2	7.2
120	63.5	404.0	2.8

The results of one experiment are given in Table V, and a graphic representation is shown in Fig. 5, where the experiment was begun at  $38^{\circ}\text{C}$ ., and the temperature was allowed to rise at first at a rate of  $2^{\circ}\text{C}$ . in six minutes, later somewhat more slowly. From  $38^{\circ}$  to  $50^{\circ}\text{C}$ . there is practically no change in permeability when the conductivity readings are calculated to a constant temperature ( $64^{\circ}\text{C}$ .) on a basis of a change of 2 per cent. per degree. Only after  $48^{\circ}$  or  $50^{\circ}$  is there a distinct rise in the rate of exosmosis, which rapidly reaches a maximum as the impermeability of the protoplasm breaks down. With the loss of salts from the cell the gradient of concentration lessens, and so the rate of exosmosis slows down and finally falls rapidly in spite of the increased permeability.

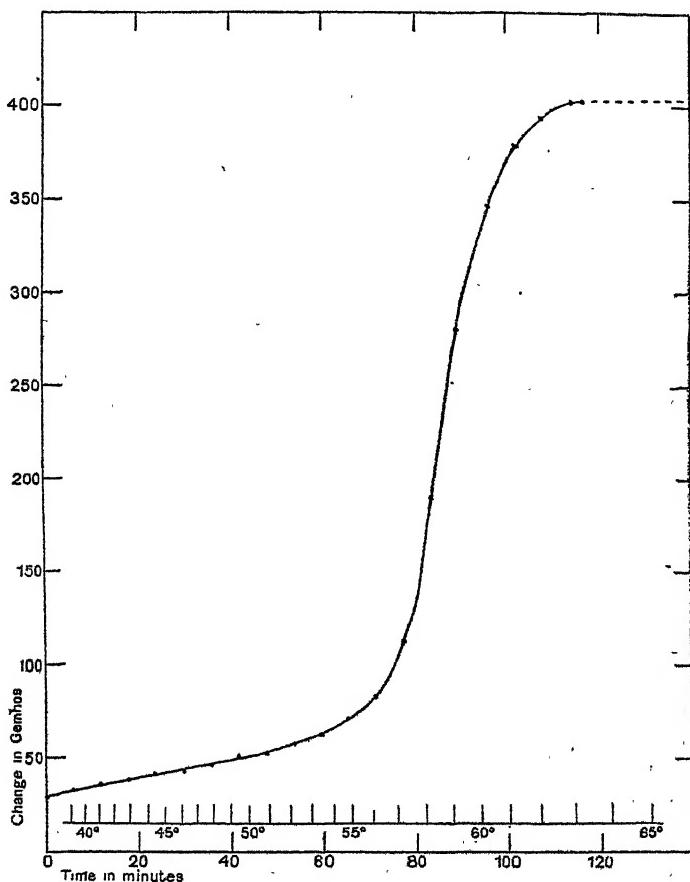


FIG. 5.

Of course in such experiments as these, where the temperature is above what may be termed normal, the time factor plays a large part. Experiments with different rates of rise of temperature would thus give curves of

a different shape. The experiments, however, seem clearly to indicate that in the case of the pulvinus of *Mimosa*, the temperature coefficient between 35° and 50° C. for the rate of exosmosis of electrolytes into water is not markedly greater than unity.

#### SUMMARY.

An excised pulvinus of *Mimosa pudica* when placed in warm water with its internal tissues freely exposed exhibits, on stimulation, repeated contractions during many hours. This indicates very clearly that the loss of turgor in the cells of the lower half of the pulvinus, which is associated with contraction, cannot be explained, as has been suggested, by a sudden increase of permeability of the tissues allowing of a rapid exosmosis of osmotic substances.

By placing a pulvinus in a very small quantity of water in a specially constructed conductivity 'cell' the exosmosis of electrolytes can be studied by the change of the electrical conductivity of the fluid. By this means it has been shown that contraction is associated with an increase in the rate of exosmosis of electrolytes from the pulvinus. The exact nature of this increase is obscure, but the increase is far too small to account for the sudden loss of turgor of the cells. The loss of turgor is probably due to the disappearance or inactivation of a considerable part of the osmotic substances of the cells.

The 'conductivity method' being direct is much superior to the indirect plasmolytic method used by previous workers for the study of the effect of light on permeability. Using the 'conductivity method' it can be shown that in the case of the pulvinus of *Mimosa* the permeability of the cells for electrolytes is markedly increased by exposure to light, but the effect rapidly falls off in time. A sudden change from light to darkness also increases permeability.

A curious autonomous (autogenic) contraction of the pulvinus was observed. In some cases, after the pulvinus had been stimulated by cooling or being brought back into warm fluid, it exhibited a series of contractions (in one case sixteen in number) although the external conditions remained perfectly constant. The contractions occurred at gradually decreasing intervals, so that the time for recovery becoming less and less the contractions also decreased in extent, and the pulvinus at the end of the series remained in the contracted state. The pulvinus of *Mimosa* thus for a time showed movements as autonomous as those of *Desmodium gyrans*.

Below 50° C. a slow rise of temperature appears to have little effect on the rate of exosmosis into water of electrolytes from the cells of the pulvinus of *Mimosa*. The increase of permeability with higher temperatures is apparently due to lethal, no doubt irreversible, changes.

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# Further Notes on Intrafascicular Cambium in Monocotyledons.

BY

AGNES ARBER.

With four Figures in the Text.

In a recent number of the 'Annals of Botany' I drew attention to the widespread occurrence of intrafascicular cambium in Monocotyledons.<sup>1</sup> I have since observed it in several additional cases, including an example from each of four families (Araceae, Dioscoreaceae, Iridaceae, and Potamogetonaceae) in which, apparently, it has not hitherto been noticed. The object of the present note is to record these cases and to enumerate the cohorts and families in which instances of intrafascicular cambium have up to the present been observed.

*Acorus calamus*, L. (Araceae). In the Sweet Flag transverse sections close to the apex of the rhizome show cambial activity within the bundles very clearly. The lignified protoxylem and the immature metaxylem are separated from the phloem by radial files of cells (Fig. 1).

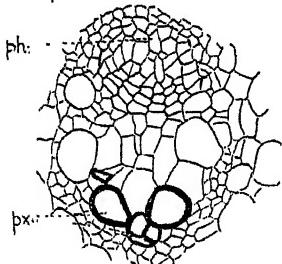


FIG. 1. *Acorus calamus*, L. Transverse section of single bundle from near apex of rhizome, showing radial rows of elements between xylem and phloem (ph.). Only the protoxylem (px.) is at present lignified. ( $\times 320$ .)

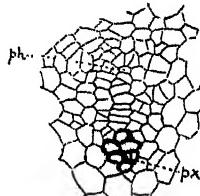


FIG. 2. *Tamus communis*, L. Transverse section of single bundle close to apex of aerial climbing stem, showing radial rows of elements between protoxylem (px.) and phloem (ph.). ( $\times 320$ .)

*Tamus communis*, L. (Dioscoreaceae). In the Black Bryony transverse sections close to the apex of the growing shoots reveal, within the individual bundles, a radial arrangement of the cells between the phloem and the lignified protoxylem (Fig. 2), but this indication of cambial activity is highly

<sup>1</sup> Arber, Agnes : On the Occurrence of Intrafascicular Cambium in Monocotyledons. Ann. Bot., vol. xxxi, pp. 41-5, 3 Text-figs., 1917.

ephemeral. It was only observed in sections so close to the apex that the diameter of the axis was about 1 mm., or, excluding the prominent leaf-bases, 0·5 mm.

*Tritonia*, garden hybrid (Iridaceae). Traces of cambium were observed in the bundles of the very young inflorescence axis, at a stage when the diameter of the axis was less than 0·75 mm.

*Potamogeton natans*, L. (Potamogetonaceae). In this species, evidence of very slight and irregular cambial activity has been found in the vascular bundles of the leaf. The same thing has been noticed in the leaves of another species, probably *P. lucens*, L.

*Ophiopogon japonicus*, Ker-Gawl. (Liliaceae). In transverse sections of the inflorescence axis, the bundles are seen to be arranged in a ring. At a very young stage there are clear indications of cambial action. The radial rows of elements within the bundle in some cases involve xylem as well as phloem.

*Phormium tenax*, Forst. (Liliaceae). Both the xylem and phloem of the mature leaf-bundles show indications of cambial activity.

*Veratrum album*, L. (Liliaceae). In the mature leaf the arrangement of the phloem elements in radial files is particularly striking.

*List of Cohorts and Families in which one or more cases of intrafascicular cambium have been recorded.<sup>1</sup>*

PANDANALES	Typhaceae
HELOBIAE	Potamogetonaceae
	Scheuchzeriaceae
GLUMIFLORAE	Gramineae
	Cyperaceae
PRINCIPES	Palmae
SPATHIFLORAE	Araceae
FARINOSAE	Commelinaceae
LILIIFLORAE	Liliaceae
	Dioscoreaceae
	Iridaceae
SCITAMINEAE	Musaceae
	Zingiberaceae
MICROSPERMAE	Orchidaceae

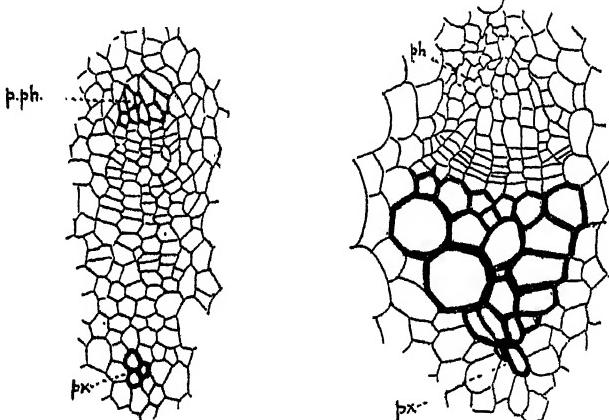
It follows from this list that intrafascicular cambium is now known from all but two of the cohorts into which Engler divides the Monocotyledons; the exceptions are the Triuridales and the Synanthes. The Triuridales consist of a small group of tropical saprophytes, which are not avail-

<sup>1</sup> The references to the literature in connexion with these records will be found in my previous paper, already cited.

able in this country, while suitable material of the few genera, such as *Cyclanthus* and *Carludovicia*, which form the small cohort of the Synanthae is also difficult to obtain.

It is probable that the list of cases of intrafascicular cambium in Monocotyledons may eventually be almost indefinitely extended;<sup>1</sup> the most favourable place for detecting the cambium in species where it is ephemeral appears to be in the young stems at a very short distance from the growing apex, but the young leaves are sometimes equally suitable.

For comparison with the vestigial cambium of the Monocotyledons I have included two drawings (Figs. 3 and 4) showing the young and



FIGS. 3 and 4. *Thalictrum flavum*, L. Fig. 3. Transverse section of young bundle from an inflorescence axis gathered June 1, 1915, in which only protoxylem (*px.*) and protophloem (*p.ph.*) are fully differentiated. Fig. 4. Transverse section of mature bundle from inflorescence axis gathered August 23, 1916.  $\times 320$  (*circa*).

mature bundles of the inflorescence axis of *Thalictrum flavum*, L. (Ranunculaceae). The tendency towards a V-shaped xylem, and the fact that the cambial activity is chiefly directed to the production of phloem, are features which distinctly recall some of the Liliaceae.

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BALFOUR LABORATORY, CAMBRIDGE.

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<sup>1</sup> That the number of known cases might be much increased by further research has been suggested by Salisbury, E. J., Science Progress, vol. xii, pp. 41, 42, 1917.



# Chondriosomes and the Primordia of Chloroplasts and Leucoplasts.

BY

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With Plate I.

IN the cytoplasm of the cells of many plants, representing all the great groups of the Vegetable Kingdom, small rod-shaped or granular bodies have been described under the name chondriosomes. It has been claimed that these bodies are readily observed in living cells of certain plants, but, in general, they are to be definitely demonstrated only after the application of certain fixing fluids and stains. The fact that bodies designated by the term chondriosome are not visible after the application of some of the best and more commonly known cytological methods used in the study of nuclear phenomena accounts for their comparatively late discovery by botanists. However, the rather long list of papers that have appeared during the last decade is an indication of the lively interest taken in the study of these bodies, which are so numerous and conspicuous in certain parts of many plants.

From a perusal of the literature, one is convinced that the several investigators have applied the term chondriosome to markedly different objects in the cell. This is definitely pointed out by Cavers (1914), who, in a careful review, has summarized the literature appearing prior to the year 1914. A discussion of the literature will, therefore, be unnecessary here, and the reader is referred to the above review by Cavers, where a complete bibliography of the literature then existing will be found.

## METHODS.

After some experience I have found it very desirable to make some modifications in the use of certain methods recommended by others for the study of the various bodies described under the general term chondriosome;

and, since others may decide to use the processes followed by me, it may be worth while to give a somewhat detailed statement of the methods employed.

Different killing fluids were used at first in fixing material, but the following proved to be so much more satisfactory for the tissues studied that it was finally employed exclusively:

1 per cent. chromic acid . . . . .	17 c.c.
2 per cent. osmic acid . . . . .	3 c.c.
Glacial acetic acid . . . . .	3 drops.

In many instances the glacial acetic acid was omitted altogether, although the cell contents, especially the nucleus, seemed to be brought out more clearly and definitely with the small quantity of the acetic acid. No satisfactory results were obtained when the amount of acetic acid in the above mixture was increased to one cubic centimetre. It is seen that one of the best combinations of these three acids used in fixing cells for the study of nuclear phenomena is quite unsatisfactory for the demonstration of chondriosomes. While the fixing of nuclei with the above mixture gave good results for some tissues, yet, in others, the details were not so well brought out. This was especially noticeable with nuclei in the resting condition. Less difference was observed in mitotic stages.

In the literature one finds listed reagents which are suitable for chondriosomes, and others in which these bodies are dissolved. In some cases the statements of different writers seem to be contradictory. It is not easy to understand this unless the different authors refer to different structures under the same name; or it is probable that the killing reagent which would preserve these bodies in one plant would dissolve them in another or render them incapable of being stained. I am inclined to believe from my experience, although I have not been able to test the matter thoroughly, that in some plants the bodies that may reasonably be placed in the category of chondriosomes are not injured by the amount of acetic acid contained in the chromo-osmo-acetic mixture used in the study of nuclear phenomena.

Specimens were allowed to remain in the fixing fluid from thirty-six to forty-eight hours. After fixation the procedure varied with the method of staining to be employed. In all tissues studied by me two methods of staining were followed, the one used as a check upon the other, and in all cases the results were essentially the same. These two staining processes consisted, respectively, of a modification of Benda's crystal violet, and the well-known iron-alum-haematoxylin stain.

*Staining with Crystal Violet.*

If the crystal violet is to be used, the procedure is as follows :

- a. Wash in water one to two hours.
- b. Equal parts pyroligneous acid (rect.) and 1 per cent. chromic acid, twenty-four hours.
- c. Two per cent. aqueous solution of potassium bichromate, twenty-four hours.
- d. Wash in water twenty-four hours.
- e. Dehydrate very carefully and bring into paraffin.
- f. Treat the sections on the slide with the iron mordant  $((\text{NH}_4)_2\text{Fe}_2(\text{SO}_4)_4 \cdot 24\text{H}_2\text{O})$ , twenty-four hours. Instead of a 4 per cent. solution, only 3 per cent. was used.
- g. Slides are now rinsed with water.
- h. Treat with alizarin sodium sulphonate (Kahlbaum). This stain is made by adding from one to several cubic centimetres of a saturated solution in 70 per cent. alcohol to 80 to 100 c.c. of water.
- i. Pour off with water and let dry in the air, standing slide on end.
- j. Stain with Benda's crystal violet by warming to the point of forming vapour.
- k. After cooling, pour off with water and let dry as above.
- l. Destain with a 10 per cent. solution of acetic acid under microscopic control. This requires from a few seconds to as many minutes, depending upon the character of the tissue and the thickness of the section.
- m. When the desired stain is reached, pour off with water and dehydrate quickly with absolute alcohol. The alcohol is immediately followed with clove oil-orange G, which is allowed to remain until the cell walls begin to show a slight orange colour, or until the desired counterstain is reached.
- n. Now pour off the clove oil-orange G with xylol and add cedar oil, which is followed by Canada balsam and a cover-glass.

The primordia of leucoplasts and chloroplasts and the chondriosomes are stained blue. Although I have used the alizarin of several times the strength of Benda's formula, this stain imparts only a pale rose colour to the nucleus, or no effect is noticeable. This stain is unnecessary. The crystal violet was prepared as follows as given by Benda :

Saturated solution of crystal violet in 70 per cent. alcohol, one part ; 10 per cent. HCl in 70 per cent. alcohol, one part ; aniline water, two parts.

*Staining with Iron-Alum-Haematoxylin.*

- a. After fixation, as in the preceding process, the specimens are washed in water twenty-four hours (in flowing water, or in several changes).

- b. Dehydrate and bring into paraffin.
- c. Sections on the slide remain in the mordant (3 per cent.) four to six hours.
- d. Rinse with water and stain twelve hours or longer in a one-half per cent. aqueous solution of haematoxylin:
- e. Destain with a 3 or 4 per cent. solution of the iron salt, after which wash or let stand in water fifteen minutes. Dehydrate and treat with clove oil-orange G. When the desired counterstain is reached, remove the oil with xylol, and mount in balsam, either with or without following the xylol with cedar oil. Care should be taken not to let the clove oil-orange G act too long, or the smaller chondriosomes may be rendered indistinguishable.

It will be seen that the latter process is shorter and simpler than the former. In some tissues prefer it to the crystal violet method.

#### OBSERVATIONS.

This paper will deal with the results obtained in the study of root-tips of *Pisum sativum*, *Zea Mays*, the thallus of *Marchantia polymorpha*, *Anthoceros*, *Pallavicinia*, the seedling of *Pinus Banksiana*, the stem and leaves of *Elodea canadensis*, and the root-tip of *Adiantum pedatum*, together with certain Algae.

*Pisum*. I began first with the root of the pea in order to verify observations of others and to test the usefulness of the methods employed. Sections were cut from three to five microns in thickness.

*Pisum* proved to be one of the most satisfactory objects for the demonstration of the transformation of primordia into leucoplasts. In the meristematic cells of the root, at the juncture of the root-cap and the tip of the root proper, one finds the structure shown in Plate I, Fig. 2. The most conspicuous objects in the cell, apart from the nucleus, are the numerous black rods (iron-alum-haematoxylin being used) occurring in relatively large numbers. These rods vary in length and thickness; they may be straight or variously curved or bent, while some present a lumpy appearance as if composed of granules. In this region of the root some of the rods show a globular thickening at one end. The rods sometimes show a tendency to accumulate about the nucleus and arrange themselves radially from it, but, as a rule, they are promiscuously scattered within the cytoplasm.

In addition to the large rods (Fig. 2) are numerous very small globular or granular bodies and very slender, delicate rods that stain the same colour and seem to be of the same ultimate composition as the large rods. The granules occur singly or in rows or chains of two or more. The very

slender, delicate rods vary also in length and thickness. In fact, all of the bodies mentioned vary in form and size. Although under lower magnification the cells appear as if containing numerous rod-shaped bacteria, the rods and granules are very readily distinguished from bacteria, because of the uniformity in the size of bacteria and the smooth, clear-cut surface of their cell walls.

Passing back in the root to where the cells of both plerome and cortex begin to elongate, it is clearly seen that the larger rod-shaped bodies change gradually into those which take on the form of a club- or pear-shaped body or that of a hand-mirror (Fig. 3). These are leucoplasts, and they contain one or more inclusions or starch grains. The starch grain, which represents the glass of the hand-mirror, remains colourless or may appear a pale blue, especially when crystal violet is used or if the destaining is not carried too far in the iron-alum-haematoxylin preparations. The protoplast, which corresponds to the handle of the mirror, stains a jet black (Figs. 3 and 4). It is quite evident, therefore, that the larger thick rods are the primordia of leucoplasts. A comparison of Figs. 2 and 3 will show how these primordia, of whatever length, are changed into leucoplasts. The transition takes place as follows: The rod becomes swollen or enlarged at one end, where a small and palely-staining starch inclusion is forming. The inclusion enlarges, becoming round or oval. The result is that the shorter rods become club- or pear-shaped leucoplasts, while the more elongated forms take the form of a hand-mirror. I do not regard the hand-mirror form as a division stage of the leucoplast. Fig. 3 shows also how numerous are the very delicate and slender rods and granules which do not become leucoplasts but retain their identity even in older cells of the root. These I shall designate as chondriosomes. Fig. 4 illustrates, more highly magnified, some of the commoner forms of the larger leucoplasts found in older cells of the root that have become greatly elongated. In Fig. 4, *b*, the leucoplast contains three smaller starch grains. At *c* (Fig. 4) a large, oval starch grain is near the middle of the leucoplast. Fig. 4, *d*, *e*, shows other forms of leucoplasts with large starch grains, while at *a* are shown a few of the very much smaller granules and rods that do not become leucoplasts. In these same cells are frequently found smaller and more rounded leucoplasts containing one or more rounded starch grains, as figured at *a*, Fig. 11, for *Pinus*. These small leucoplasts are more difficult to stain than the larger and may be readily overlooked. In addition to the foregoing leucoplasts, the most conspicuous phenomenon in all cells of the root-tip is the presence of innumerable round or rod-shaped chondriosomes. These bodies do not form the groundwork of the cytoplasm, the latter being a much more finely granular or homogenous substance.

Passing now from the root-tip proper to the root-cap, we note that the

rods become fewer, but there seems to be little or no falling off in the number of granules. Fig. 1 represents a cell from near the end of the root-cap, in which rounded starch grains are present in the distal end of the cell. The most conspicuous objects in the cytoplasm, even when examined with the ordinary high powers of dry lenses, are the numerous chondriosomes in the form of densely-staining granules, rows of granules, and rods. These objects, so numerous here as elsewhere, far exceed the number of starch grains that could be contained in any cell of the root-cap, and the conclusion is that they are not identical with leucoplasts. While it is not possible to distinguish between the primordia of leucoplasts and the rods and granules that do not become leucoplasts, I have come to the conclusion, from the presence and behaviour of all these bodies in both young and older cells, that the primordia of leucoplasts are not the same as these other objects. This conclusion is borne out in the case of all other plants to be noted below, in which it will be shown that the primordia of chloroplasts are identical with those of leucoplasts and that it is probably better to confine the name chondriosome to the objects that do not give rise either to leucoplasts or to chloroplasts. Both chondriosomes and primordia of leucoplasts multiply by transverse division or constriction.

The rounded starch grains of the root-cap reveal the plastid as a narrow, darkly-staining crescentic or rounded body at one side, although the plastid doubtless surrounds the whole starch inclusion. These starch grains were developed from the smaller rounded or rod-shaped plastids. Lewitsky (1910) figures a cell from the root-cap of *Asparagus officinalis* (his Fig. 18) in which similar starch grains are shown, but these bodies are designated in the description of the figure (1910, p. 546) as 'Statoblasten'. In the drawing he does not show the plastid. While some of the leucoplasts in the root-cap and in other parts of the root, as in the cortex, are derived from rounded primordia, yet the transition of the short rod through the club- and pear-shaped forms is easily observed.

In the elongating cells of the vascular cylinder of the root, the primordia of leucoplasts are apparently more numerous and longer than in cells of the cortex. All of the primordia do not develop starch inclusions; those that do not become long and frequently thread-like. They finally undergo disorganization in older elements. In the cortex of the root the primordia seem to be generally shorter than those of the central cylinder.

*Zea Mays.* As a second phanerogamic root-tip, that of *Zea* was chosen.

In the meristematic cells of the tip, including those of the root-cap, or calyptrogen, are numerous densely-staining granules and rods somewhat evenly distributed in the groundwork of the cytoplasm. These bodies are smaller than in the root of *Pisum*. The granules are by far the more

numerous. Some of the granules present a smoother contour than others. The rods vary in length, and to some extent in thickness. Some are club-shaped and some dumb-bell-shaped. In cells of the root-cap the rounded granules far exceed the rods in number as in *Pisum*. In the older parts of the root-cap, where the cells have begun to round off and separate from each other, rounded leucoplasts with their starch inclusions are present and confined chiefly to the distal ends of the cells, although a few are distributed throughout the cell. The transition of the short rod-shaped primordia, through club-shaped, pear-shaped, and hand-mirror-like forms, so conspicuous in *Pisum*, is not so easily observed in the root-cap of *Zea*. Some of the leucoplasts arise from rounded primordia, these appearing at first only slightly larger than the densely-staining granules, but with a sharp contour or rim and a colourless, or only slightly stained, centre. In *Zea* such very small leucoplasts are present in small numbers in the meristematic cells. In cells of the dermatogen and plerome, the transformation of rod-shaped primordia through the forms just mentioned is conspicuous. As in *Pisum*, there are also present, in addition to the primordia, the very numerous rounded chondriosomes. They persist in large numbers even in older cells. The behaviour and fate of both plastids and chondriosomes is the same as that described for *Pisum*.

*Marchantia*. No other plant selected by me for study shows more clearly the sharp and well-defined difference between chloroplasts and the rod-shaped or granular bodies which are not chloroplasts and which do not develop into chloroplasts, but which reveal and retain their identity in both young and old cells.

In Fig. 5 we have a longitudinal vertical section through the growing point of the thallus, including one apical cell and two young ventral scales. In the apical cell, and in those touching it, are to be seen the young rounded chloroplasts and the very small rod-shaped and granular bodies. The groundwork of the cytoplasm is illustrated by fine stippling. In older cells, removed two or three layers from the apical cell, the difference between the rounded chloroplasts and the rod-shaped chondriosomes is more obvious. Fig. 6 is a cell taken a little farther back from the growing point than those in Fig. 5. Here some of the chloroplasts appear more rounded and larger, probably with a flat side turned towards the observer. Others are narrower and lenticular in shape with a light strip running lengthwise through the middle. In this position the chloroplasts present a view not unlike the flattened side of a grain of wheat or a coffee bean (see also Fig. 6). The chloroplast in these cells seems, therefore, to be a flattened object, round, oval, or elliptical when viewed from the flat side, but somewhat lenticular when seen from the edge. In older parts of the thallus, the colourless, lenticular centre is, doubtless, an inclusion of starch. In some instances the chloroplasts in older cells present the appearance of Fig. 14, a,

in which there seems to be more than one starch inclusion. This is especially true in young gemmae, in certain vegetative cells of the antheridiophore, and elsewhere. Such chloroplasts probably contain two or more starch grains or small masses of starch, and in this respect chloroplasts and leucoplasts seem to be alike. The nucleus (*n*, Fig. 6) has a very delicate nuclear membrane, with the chromatin in the form of small, rounded granules, as may be seen also in cells of Fig. 5. Fig. 7 presents a cell beneath a dorsal air-chamber. In the living thallus such cells appear green with numerous large, well-developed chloroplasts. The chloroplasts here stain a deep blue with the crystal violet and present a uniform, homogeneous structure. This is the usual behaviour of mature chloroplasts rich in chlorophyll. In these and similar cells (Fig. 7) the chloroplasts show the usual division stages. In such cells the chondriosomes likewise show division stages (Figs. 6 and 7). If we now turn to still older cells in the thallus, we have the structure presented in Fig. 8, which represents a tangential view of the cell. The chloroplasts are smaller and irregularly distributed along the periphery of the cell. The scanty cytoplasm is omitted from the figure. They are rounded or somewhat lenticular, as described above. The majority of the chondriosomes are in the form of delicate rods of varying length. Some seem to be dividing. In preparations stained with iron-alum-haematoxylin, the chondriosomes appear black, while with the crystal violet their colour is a beautiful deep blue. In such cells the chondriosomes stand out so clearly that the preparations make most excellent objects for the demonstration of both the chloroplast and the chondriosome.

Taking the thallus of *Marchantia* as a whole, we find chondriosomes in all cells. In the apical cells and their immediate neighbours, the chloroplasts and their primordia are small, but, as a rule, readily distinguished from the smaller granular or rod-shaped chondriosomes. In the isodimensional cells not far removed from the growing point, the chloroplasts begin to assume their adult form, being much larger. The chondriosomes are, however, larger and more sharply defined than at the growing point, but always in the form of granules or rods. Many are seen in process of division. Short rows of granules are found here also. In the dorsal chlorenchyma layer, chondriosomes are relatively few, but they are thicker than in deeper parts of the thallus. Those of the cells forming the floor of the dorsal air-chambers are relatively larger than in other cells below them (Fig. 7). The older cells near the centre of the thallus have apparently few chloroplasts, but many slender, rod-shaped chondriosomes that vary greatly in length, along with those that may be spoken of as very short rods or granules. Many of these rods and granules show constrictions in the middle as if in the process of division (Fig. 8). The division stages in younger cells present the same appearance. In much older parts of the

thallus, chloroplasts and all other contents of the cell present conclusive evidence of disorganization.

It is, however, in the mucilage-producing hairs borne by the ventral scales and springing from the floor of the gemma cups, and in the young rhizoids, that chondriosomes find their greatest development. The large mucilage hair (Fig. 5, *a*) presents the usual structure of such cells at that age. In the rather dense cytoplasm the numerous rods and granules are strikingly conspicuous. Many of the rods are long, exceeding the greater diameter of the nucleus. Some of the rods show clearly that they consist of a row of very small granules. In the younger mucilage hair (Fig. 5, *b*) the cytoplasm is very dense, and the chondriosome primordia are smaller. There are no chloroplasts in the larger hairs, and in the youngest all staining bodies distinguishable in the uniform groundwork of the cytoplasm are minute. Whether chloroplast primordia are present here, I am not able to say with certainty. The cells of the young ventral scales bearing such mucilage hairs show the usual chloroplasts and chondriosomes, which are clearly and sharply differentiated.

Perhaps one of the most striking parts of the thallus is seen in the young rhizoids (Fig. 9). The chondriosomes are very large and extremely numerous. In thick sections the whole cell seems to be filled with the blue or black rods and granules. These rods and granules show the characteristic division stages. No recognizable chloroplasts are evident at this stage of the hair's development, although the cells of the ventral epidermis, from which the hairs develop, contain chloroplasts. Whether any of the bodies shown in Fig. 9 are disorganizing chloroplasts I am unable to state. As the rhizoids mature, all cell contents gradually disappear.

The mucilage hairs arising from the base of the gemma cups and in the antheridial cavities do not contain chloroplasts but very numerous chondriosomes, although these are smaller than in the mucilage hairs borne by the ventral scales and by the young rhizoids.

The methods of fixing and staining used here bring out clearly the progress in the formation of mucilage or slime in the so-called mucilage-bearing cells of the thallus of *Marchantia*. The mass of slime first appears in the centre of the cell. It presents a very fine, uniformly granular appearance, staining a greyish, bluish, or brownish colour, depending upon the intensity of primary staining with the haematoxylin or violet and the counterstain with the orange G. As the mass of mucilage increases, the cytoplasm, together with the nucleus, chloroplasts, and chondriosomes, are crowded into a thin layer along the cell wall. The entire living content of the cell is finally used in the formation of the slime.

The mucilage excreted by mucilage hairs in both gemma cups and antheridial cavities, for example, seems to be of quite a different composition. In the latter case, no trace of mucilage can be seen inside these

hairs. The mucilaginous substance surrounding the young gemmae stains a beautiful light brown with the orange G. It is a perfectly homogeneous and structureless mass.

In all developmental stages of a gemma, chondriosomes, along with the chloroplasts, are present in the cells. The large stalk cell of the gemma contains relatively few chloroplasts, but the chondriosomes are conspicuously numerous.

In the early stages of antheridial development, the chloroplasts are eliminated from the central cells that give rise to the spermogenous tissue, being confined to the wall of this organ. In the wall cells both chloroplasts and chondriosomes are present, but only the latter in the spermogenous tissue. In all stages of antheridial development, chondriosomes are numerous and conspicuous in the spermogenous tissue up to the time of the final and diagonal division of these cells. They are in the form of granules or very short rods. Their fate was not traced in the transformation of the cell into the sperm following the diagonal division.

*Anthoceros.* Inasmuch as the cells of *Marchantia* contain a number of chloroplasts, the question naturally arose as to whether the chondriosomes were not merely products of the disorganization of chloroplasts, or, in meristematic cells, undeveloped primordia of chloroplasts. In order to compare the chondriosomes described in the foregoing for *Marchantia* with those of a plant with one chloroplast to the cell, a study of the thallus of *Anthoceros laevis* was made. Scherer (1913, p. 497) states that during the entire development of the gametophyte of *Anthoceros Husnoti* every cell, with the exception of the apical cell, possesses one chloroplast and a larger or smaller number of chondriosomes.

My observations were made on the gametophyte of *Anthoceros laevis*. In all parts of the plant studied I am able to confirm the results of Scherer and to extend his conclusion to the apical cell as well. In the apical cell (Fig. 13), as in other vegetative cells of the thallus, the chondriosomes are very small and difficult to demonstrate, appearing, for the most part, as very slender and delicate rods or granules. They are relatively smaller than those of *Marchantia* and fewer in number in the ordinary cells. In the cells of the wall and stalk of the antheridia, on the contrary, the chondriosomes are larger and more conspicuous and retain the stain with greater avidity. I am also able to confirm the observations of Scherer in regard to the presence of chondriosomes in the spermogenous tissue and in the egg cell of the archegonium. The chondriosomes as figured by Scherer (l. c., Fig. 9) for cells of the spermogenous tissue are quite similar to those mentioned in the foregoing for *Marchantia*. In an early stage of the transformation of the sperm, a conspicuous rod is seen in each cell along with the nucleus. This I interpret as the blepharoplast. I was not able to convince myself that chondriosomes were present in such cells.

Since there are always several chondriosomes in spermogenous cells at earlier stages in the antheridium, it is extremely doubtful whether the blepharoplast, which is preserved in fixing solutions containing acetic acid, represents a chondriosome.

The fear that chondriosomes may be products of disorganized chloroplasts disappears entirely in the case of *Anthoceros*, since each cell reveals its conspicuous chloroplast, in which there appears no sign of disorganization.

It may be worthy of note that the technique, as here employed in the study of chondriosomes, is well suited to bring out the structure of the chloroplasts, together with their contained collection of rod-shaped or granular pyrenoid bodies described by McAllister (1914). The pyrenoid bodies, in form and staining reaction, bear some resemblance to the chondriosomes which are present in the stalk cells of the antheridia. These pyrenoid bodies are, as a rule, larger and stain more densely than chondriosomes found in the vegetative cells of the thallus.

*Pallavicinia*. In the cells of this liverwort, which contain many chloroplasts, chondriosomes are present, especially in the meristematic region, although they occur also in the large cells some distance removed from the growing point. In form they are similar to those of *Marchantia*, being, however, smaller and less numerous. In the large apical cell, in which the relatively small chloroplasts stand out with striking clearness, the chondriosomes are few, and in the form of small rods that do not stain deeply. As in the apical cell of *Anthoceros* they are difficult to bring into evidence and may readily be overlooked. In the middle of the thallus, about four or five cells back of the apex, small round or globular chondriosomes were found to be numerous in some preparations. In the older cells of the thallus they appear, when stained with crystal violet, as very pale blue slender rods or threads. The paucity of chondriosomes in the hair-like outgrowths of the growing end of the thallus presents a striking contrast to the large numbers found in similar structures in *Marchantia*.

*Adiantum*. In this plant the following statements will be confined to the results obtained in the study of the root-tip, while a discussion of the presence and behaviour of chondriosomes and plastids in other organs of the fern will be reserved for a future publication. The tips of fresh young roots about one centimetre long that had developed directly from the growing end of the stem were selected for study.

In the apical cell there are to be recognized two apparently different and distinct primordia: small granules and rods that stain densely and uniformly, and larger bodies which are chiefly lenticular in shape. The former correspond to chondriosomes, as used in this paper, and the latter are clearly the primordia of the leucoplasts (Fig. 13). These leucoplast primordia present the same form and structure as certain chloroplasts in

the liverworts and other plants studied, being, as stated, lens-shaped with a colourless or less densely stained lenticular centre, especially after staining with crystal violet. If vacuoles be present in such cells, both classes of bodies are mainly aggregated about the nucleus.

Passing from the apical cell into the root-cap, it is seen that in the young cells of the root-cap, namely, the last segment cut off, or the two or more cells resulting therefrom, the primordia of leucoplasts are perceptibly larger and stain densely. In the third and fourth layers of the root-cap, they show a further increase in size, and, in crystal violet preparations, the pale or colourless lenticular centre is more pronounced. In the fifth layer the typical cell contents are shown in Fig. 14, *b*. The leucoplasts are usually lenticular or elliptical in shape. The almost colourless lenticular centre gives the impression that these bodies are hollow or contain an inclusion which stains only slightly. The chondriosomes consist mostly of small granules or short and more delicate rods.

In this layer of cells of the root, some of the leucoplasts are seen to contain two lenticular inclusions. In the sixth and seventh layers of the root-cap in question, all leucoplasts stain poorly with both the haematoxylin and the crystal violet. Each contains from one to three or four lenticular inclusions, as shown in Fig. 14, *a*. In the eighth and outermost layer of this root, the cells are beginning to separate from each other, and signs of disintegration are very apparent. The leucoplasts and their inclusions have disappeared from certain cells, but even in these the small granular and rod-shaped chondriosomes are very numerous; they are among the last of the protoplasmic contents to disappear from these cells.

In the cells of the root-cap, as well as in many of those of other parts of the root, especially in the cortex, there is present a cluster, or clusters, of rounded bodies of varying sizes which do not take the violet or haematoxylin stains readily, but which stain a pale orange with the clove oil-orange G (Fig. 14, *b*). As nearly as can be determined, these clusters seem to be plastids with partly digested starch inclusions. In many of the cells of the cortex and older parts of the plerome cylinder these pale orange-coloured clusters are conspicuous, appearing in contrast with the larger plastids with their inclusions, which vary in colour from a pale blue to a light or smoky grey in preparations rather densely stained with haematoxylin. In many cells of the outer cortex, as we pass back into the older parts of the root, the pale orange-coloured bodies are the only objects of the cytoplasm present in addition to the chondriosomes. The probability that they are the products of the plastids is strengthened by the fact that they are among the last structures to disappear in disorganizing cells of the root-cap and in those of the older parts of the root.

Passing back from the apical cell to the body of the root, we find, in the latest segments cut off, that the primordia of the plastids and chondriosomes

are of the same size and structure as in the apical cell. In the cells resulting from the second or third older segments, the primordia, now larger and more numerous, stain very densely. In slightly older cells some become club- and dumb-bell-shaped. In cells of the central cylinder, a little farther back, the densely staining rods are the most strikingly conspicuous objects in the cell. This is well illustrated in Fig. 15. Here it is seen that the rods vary in length, many being variously curved or bent. In addition to the rods, which are unquestionably derived from the primordia of plastids seen in the apical cell, there are the ever-present granules in countless numbers. They occur singly, in pairs, as if in division, and in chains. These granules do not constitute the groundwork of the cytoplasm (Fig. 15). In the narrow elements of the central cylinder of the root, which have dense cytoplasm, the rods are numerous and especially conspicuous, because they are thicker and stain densely. Many seem to be undergoing transverse division, but whether they break up into the small granules cannot be stated with certainty. The leucoplasts in the form of a hand-mirror, which are rather conspicuous in *Pisum*, are very rare in the fern and of a much smaller size.

In the wider elongating cells of the central cylinder, which have sparse cytoplasm, the rod-shaped primordia of leucoplasts that fail to develop inclusions become greatly elongated and flattened threads staining a pale blue (Figs. 16, 17). They are undergoing disorganization, for in older cells they disappear. Figs. 16 and 17 represent tangential views of portions of two large cells of the central cylinder. In addition to the long and disorganizing threads are the very numerous small rods and granules, the chondriosomes, which are present in younger parts of the root and which do not seem to be derived from the plastids nor from the conspicuous rods that develop from the plastids. In Fig. 17, at the left, is seen a cluster of starch grains which, together with surrounding cytoplasmic granules, stain a pale orange. The group is the same as those described in a preceding paragraph for cells of the root-cap.

The plastid primordia in the younger cells of the periblem and dermogen are identical with those of the central cylinder, but in this region, as the cells begin to elongate and enlarge, the primordia do not become long rods. These cells present a striking contrast, therefore, to those of the central cylinder with their numerous densely stained rods. In these periblem cells some of the primordia develop into rounded leucoplasts with starch inclusions; others remain as they appeared in the younger cells. The small, densely-staining round chondriosomes, conspicuous as black or blue dots, are present in large numbers in both old and young cells of the periblem as elsewhere. In older cells of this region there remain in the cytoplasm the yellowish-staining clusters of starch grains, a few isolated leucoplasts, pale in colour, and the numerous round chondriosomes.

It seems reasonable to conclude from the foregoing that in the growing root-tip of *Adiantum* there are two distinct organs in the cytoplasm, namely, the primordia of plastids and other bodies, which I have spoken of as chondriosomes. The latter vary in shape from spherical granules to short, delicate rods, the former always exceeding the latter in number. They divide rapidly with the growth of the cell, thereby becoming very numerous, especially in the large, rapidly elongated cells of the central cylinder. The primordia of leucoplasts, on the contrary, develop in the root-cap into bodies resembling certain chloroplasts, which contain one or more lenticular inclusions of starch. In the root proper these primordia remain in their original form as small, elliptical bodies, or develop into starch-bearing plastids, or in the plerome cylinder they may become large, elongated rods, which do not give rise to leucoplasts but continue to elongate into long-drawn-out threads which finally disappear.

*Pinus.* In the Gymnosperms, the young seedlings of *Pinus Banksiana* that did not exceed a centimetre in length supplied the material for study. In the cells of the growing point of the stem all primordia of cytoplasmic differentiations are small. Two kinds may be recognized in the groundwork of cytoplasm, which appears as a network in preparations stained with the haematoxylin method,—namely, very small round or globular bodies with a colourless centre, which may be represented by drawing a very small circle with a hard pencil, the primordia of leucoplasts and chloroplasts, and very small, densely-staining black specks, those of the chondriosomes. Passing to older parts of the seedling, as, for example, to the cortex of the young stem or to the cotyledons, we see that the circular and almost colourless primordia develop into the chloroplasts or leucoplasts, while the densely-staining bodies, which appeared as minute black specks, have become larger granules or rods, as shown in Fig. 10. I have not been able to convince myself that these minute bodies ever develop into leucoplasts or chloroplasts. The large densely-staining rods, so conspicuous in the roots (Figs. 2, 15), are not present in the parts of the seedling observed. The chloroplasts in such cells are rounded, oval, or lenticular bodies with colourless inclusions. In some the inclusion constitutes the bulk of the object; in others it is smaller, lying near one end. The form of the individual chloroplasts differs somewhat in the same and in different cells. In some cases they seem to be thinner and flatter; in others, thicker and more rounded. It cannot be stated with certainty that the lenticular form is merely an edge view of a flattened, oval, or rounded chloroplast as seems to be true in some cases. The small, densely-staining, and homogeneous granules and rods are very numerous in the cortical cells of the stem and the cotyledons (Fig. 10). Their number seems to vary considerably, however, in different cells. Both rods and granules seem to multiply actively by division, for division stages are readily observed. It seems reasonable to

believe that the rods represent rows of granules closely united, which may separate into granules. As to this, I am not thoroughly convinced. While the round bodies are much more numerous, yet rods usually occur, and some of these rods present a perfectly homogeneous structure, not only here but also in the other plants included in this study.

Deeper in the stem, near the base of the cotyledons, the chloroplasts present the form shown in Fig. 11, *a*. They are similar in form, being a trifle larger. One or two inclusions may be present. The inclusion, or inclusions, may be relatively small, occupying one end of the plastid, or larger, entirely filling it. Passing now to the outer part of the cortex of the stem, the chloroplasts are seen to be much larger, rounded or polygonal, and somewhat crowded (Fig. 11, *b* and *c*). These full-grown chloroplasts stain very densely and present a homogeneous structure, save for the presence of one or more inclusions. In the large, polygonal chloroplasts the two or more rounded inclusions are either grouped near the middle or at one side, or somewhat scattered. The smaller and rounded chloroplasts contain, as a rule, but one inclusion located at one side (Fig. 11, *b*). At *b*, Fig. 11, are shown five small rounded bodies which are derived from the small rods and granules shown in Fig. 10, namely the chondriosomes. They vary in size and in number in different cells. In cells of the enlarging cotyledons, in which large intercellular spaces have begun to be formed in the chlorenchyma, the cells, rich in large chloroplasts, contain relatively few chondriosomes. In the cells of the elongating hypocotyl, and especially those of the cortex, the chondriosomes are especially numerous.

It may be stated here that in none of the objects used in this study have I found chloroplasts in the form of the conventional rounded or elongated object with a dense body in the middle called a pyrenoid. The nearest approach to the conventional chloroplast is seen in Fig. 11, *c*, which shows a large chloroplast in division. In the cortex of the hypocotyl, division stages of the large chloroplasts are frequently met with. Deeper in the hypocotyl, the leucoplasts or chloroplasts usually present the form of Fig. 11, *a*. Along with these, there are present those that are club-shaped, or pear-shaped, or in the form of a hand-mirror.

*Elodea*. A study of the stem and young leaves of *Elodea canadensis* was made in order to compare my findings with those of other observers.

Cells of the base of young leaves present an appearance not unlike those of certain root cells of *Pisum*, *Adiantum*, and *Zea*, as shown in Figs. 2 and 15, the large, densely-staining, rod-shaped primordia of the chloroplasts being, apart from the nuclei, the most conspicuous objects in the cell. Lewitsky gives a good representation in his Figs. I and II (2, 1911). Some of these bodies are dumb-bell-shaped, as stated by Lewitsky, and are undoubtedly undergoing division. Many are also club-shaped. In addition,

there are present small globular granules, as described above for the other plants.

In successively older leaves it is quite easy to trace the transformation of these rods into the chloroplasts in passing from the base up into the leaf, and into leucoplasts, from the base of the leaf into the cells of the node of the stem. The series of changes into chloroplasts is almost identical with that described for the leucoplasts in the root of *Pisum*.

Passing into the leaf, these rods become oval, pear-shaped, or club-shaped, and many take the form of a hand-mirror. These various forms owe their existence to an increase in size and especially to the accumulation of an inclusion. The inclusion is starch, as can be readily demonstrated in the fresh leaf by the iodine test. The inclusion stains very slightly or not at all with haematoxylin. The starch inclusion may lie, for example, in the centre or in the broad, or sometimes in the narrower, end of the oval or pear-shaped chloroplast. Some are in the form of a dumb-bell with an inclusion in each end; these are division stages. Towards the tip of the young leaf, and in older leaves, the vast majority of the chloroplasts are oval or rounded, with the other described forms intermingled. In some cases the lightly-stained inclusion represents the bulk of the plastid, while the densely-staining part appears as a narrow rim thickened at one side into a crescent. This can be verified in the fresh leaf by means of the iodine test.

As the chloroplasts become older, the inclusion stains more densely, giving the chloroplast a more homogeneous appearance throughout. Many chloroplasts show two or more inclusions. It will thus be seen that the development of the chloroplast from the rod-shaped primordium corresponds, in a large measure, to the same in *Pinus*. The fully developed chloroplasts may undergo division as well as their primordia, division stages being readily observed.

In the cells of the developing leaves, in addition to the chloroplast primordia, there are present numerous very small globular and rod-shaped bodies which do not develop into either chloroplasts or leucoplasts as described and figured in the foregoing for the other plants. These bodies are usually very numerous in cells of the leaf containing fully-developed chloroplasts. They are mainly globular, but many are in the form of delicate, short rods. They stain densely and uniformly in the cells in question. To these bodies I prefer to restrict the term chondriosomes in this plant.

Passing from the base of the leaf into the nodal cells of the stem, the same transition of densely-staining, rod-shaped primordia into the leucoplasts is seen, with the difference that the change from one to the other is more abrupt. The starch grains are circular, surrounded by a very thin layer of the plastid, which, however, may be widened at one side, appearing,

if sufficiently stained, as a narrow crescent. The size and staining capacity of these starch grains vary in different preparations, depending probably upon the condition of nutrition in which the plants were when killed for study.

In the axillary scales of *Elodea canadensis* plastids are not well developed, very few chloroplasts or leucoplasts being present in the preparations. The two kinds of primordia, namely, small granules and rods, are very numerous. In certain cells of these axillary scales, some of the rod-shaped primordia develop into rather long and smooth rods or rods made of rows of small granules, which stain black with haematoxylin, not unlike those found in the mucilage hairs of *Marchantia* (Fig. 5).

In the meristematic cells of the stem-tip of *Elodea*, two sorts of primordia are present in the homogeneous groundwork of the cytoplasm, namely, very minute round bodies and slender rods. The rods are very conspicuous in well-stained preparations. As in meristematic cells of other plants, they vary in length and thickness, some being robust, and others very slender and delicate. Some seem to consist of a row of densely-staining granules. The transformation of these rods into chloroplasts and leucoplasts has been described in the foregoing. The minute round bodies stain palely and have a colourless centre. They may be represented by simply making a small circle with a hard pencil. Their periphery seems to be rather firm for so small an object, and that fact enables one to detect their presence. In older cells of both leaf and stem they are somewhat larger and very numerous, and they stain densely and uniformly. They appear now to be globules or very short rods of uniform structure; for many become elongated into short rods which may be, as a rule, about two to four times as long as thick. In older cells of the leaf with well-developed chloroplasts, these granules and short rods lie among the chloroplasts and stain like them. Both rods and granules divide by fission like mature chloroplasts or their primordia. As mentioned in an earlier paragraph, both sorts of primordia may be seen in fresh cells of young leaves that have been treated with iodine in potassium iodide solution.

*Hydrodictyon*. Space will permit of only a brief reference to findings in this plant. In cells of healthy, growing, though immature, individuals, the technique here employed revealed numerous round, or apparently globular, bodies remaining densely stained with the haematoxylin when other cell contents were colourless as a result of destaining. Since there are no individualized chloroplasts in the species in question, and the starch formed is clustered about the easily recognized bodies that pass as pyrenoids, there is no danger of confusing primordia of plastids with these bodies, which, for the time being, may be designated as chondriosomes. They are to be seen in the live cells as round, highly refractive bodies.

A further discussion will be reserved for subsequent publication.

## DISCUSSION.

A number of investigators (Guilliermond, Lewitsky, Rudolph, Forenbacher, and others) are now agreed that, in the higher plants studied by them, both leucoplasts and chloroplasts are developed from round or rod-shaped primordia which may be readily observed in meristematic tissues, either in the living state or after the application of certain definite fixing and staining procedures, and which have been referred to as chondriosomes. It is also to be inferred from the literature that leucoplasts and chloroplasts are morphologically the same—a conclusion substantiated by the facts. But as to whether these primordia of chloroplasts and leucoplasts are permanent organs of the cells, having morphological rank, there is doubtless much difference of opinion. A number of observers have confined themselves chiefly to these primordia, while others have mentioned, in some cases only incidentally, the presence of other bodies which do not develop into chloroplasts or leucoplasts. Forenbacher, who has described the transformation of chloroplasts and leucoplasts from their primordia in the stem and root of *Tradescantia*, merely mentions the fact, without further comment, that other bodies are present in the cells, which are morphologically like chondriosomes (1911, p. 658): ‘Es ist erwähnenswert, dass ich immer sowohl im Stengel wie in der Wurzel neben den schon entwickelten Chromatophoren noch Gebilde, die morphologisch mit den Chondriosomen vollkommen übereinstimmen, vorfand.’ As to the morphological individuality of the primordia of leucoplasts and chloroplasts Forenbacher is non-committal (1911, p. 660).

On the other hand, Rudolph (1912) and Sapěhin (1913) distinguish between the primordia of plastids and other bodies that do not develop into either chloroplasts or leucoplasts. Rudolph is strongly inclined to the view that chondriosomes and the primordia of plastids are entirely different structures. In his summary (1912, p. 626) he says that in the primary meristem of the growing point (speaking of the stem of *Asparagus officinalis*) there are present chiefly granules varying in size. Elongated forms are mixed in among them, occurring singly, but these may be regarded as division stages. Some of the granules increase rapidly in size, multiply, and change into chloroplasts and leucoplasts. The division stages are at times, even in older tissues, elongated so that the rod-shaped forms approach those which are filamentous in form with swollen ends. These are especially abundant in the elongated elements of the vascular bundles. However, all transitions to normal division stages are present. The remainder of the granules of the meristematic cells persist in their original form, multiply as the cells divide, and thus become distributed to all cells. Out of these are formed threads varying in length, which become more numerous as we leave the growing point. It is probable that the threads represent incompletely

divided chains of smaller granules. These structures are designated by Rudolph as chondriosomes (*Mitochondrien, Chondriokonten, Chondriomiten*). In the mature cells of pith and cortex, he continues, are to be seen side by side full-grown chloroplasts and chondriosomes without transitional stages. In the elongated cells of the vascular bundle and accompanying tissue, the long-drawn-out division stages of chromatophores may deceive one as to transitions. It seems more probable that chondriosomes and chromatophores (he doubtless means here chloroplasts and leucoplasts), though alike morphologically and in staining properties, are structures of a different nature without any genetic connexion existing between them. In no case are chondriosomes changed into chromatophores.

From the foregoing summary of Rudolph, it is clear that he recognizes two forms of primordia in the meristematic cells of the stem-tip, namely, granules and rods. He regards the rods as probable division stages. Some of the granules increase rapidly in size, multiply by division, and change into leucoplasts and chloroplasts. The long, conspicuous rods, some with swollen ends, which are numerous in the elongating cells of the vascular bundle, are also regarded by Rudolph as division stages. These he calls chondriosomes. He does not show how a leucoplast arises, and the small, conspicuous, rounded granules in these cells are referred to in his description of Fig. 6 as 'Mitochondrien, Chondriokonten und lange Chondriomiten'.

Sapéhin (1913), using the Mosses *Polytrichum*, *Funaria*, *Byrum*, and *Mnium* as objects of study, distinguishes clearly between plastids and chondriosomes, concluding that these two bodies are quite independent structures. He strongly supports the doctrine of the individuality of plastids. In almost all cells of gametophyte and sporophyte (1913, p. 323) he finds both chondriosomes and plastids. The former occur as granules or delicate, slender rods of varying length.

In the closing paragraph of this paper, Sapéhin makes the astonishing statement that in the Characeae, Bryophyta, and Pteridophyta the structures known as centrosomes and blepharoplasts are merely plastids.

Guilliermond has published numerous short papers on chondriosomes, in which are presented the results of his observations made upon various higher plants and several fungi. He applies the term chondriosome (*mitochondria, chondrioconite*) to the primordia of leucoplasts and chloroplasts and to the bodies of similar form to which he attributes the formation of anthocyanin in higher plants, and to the rods and granules in the Yeasts, and a number of other fungi, which, he asserts, give rise to metachromatin granules and fats. In his earlier papers Guilliermond (2, 3, 4) describes the formation of leucoplasts and chloroplasts from their chondriosome-like primordia. A number of his later papers (8, 10, 13, 14) are devoted to the origin of the pigments, anthocyanin, xanthophyll, and carotin of certain

plants. In one instance he refers to leucoplasts occurring in the same cell with numerous filaments or rods impregnated with anthocyanin. Referring to the epidermis of the young petals of *Iris germanica*, at a stage when the cells possess a central vacuole containing anthocyanin, with the cytoplasm and nucleus confined to a peripheral layer, he says (13): ‘Dans le cytoplasme on distingue, en dehors des chondriocentes en voie de se transformer en leuco- ou chromoplastes, de nombreux filaments ou bâtonnets imprégnés d’anthocyane.’

Guilliermond was among the first to ascribe to the bodies which he designated as chondriosomes the morphological rank of unity equal to that of the nucleus.

Anthocyanin, according to Guilliermond (13), is formed, owing to circumstances, either directly as a pigment within the chondriosome or from colourless phenol compounds by oxidation.

Löwschin (1914), in the young leaves of the rose, and Mirande (1916), in those of *Azolla filiculoides*, trace the pigment, anthocyanin, to its origin in chondriosome-like primordia, although these observers differ somewhat in regard to the precise manner in which the pigment is produced.

In certain Ascomycetes and Basidiomycetes, and in the Yeasts, numerous granules and rods are present in the cells of the mycelium and conidia (*Penicillium*), in the ascus (*Pustularia*), and in the basidia of certain auto-Basidiomycetes. In *Endomyces Magniesii* long rod-shaped bodies are found, with colourless inclusions, in the enlarged end, giving the body the form of a club, and bearing a strong resemblance to the primordia of a leucoplast with a starch inclusion. In the fungi also Guilliermond considers the ‘chondriosome comme un élément constant et indispensable de la cellule, au même titre que le noyau’ (1913, p. 1784).

Janssens and Van de Putte (1913) describe and figure numerous large rods, granules, and rows of granules in the young ascus of *Pustularia vesiculosus*, which bear a striking resemblance to the chondriosomes described in the foregoing for *Marchantia*. When the ascospores are formed, many of the rods and granules are included in them. Likewise, relatively very large rods and granules are described in the cells of the yeast *Saccharomyces cerevisiae*, by Janssens and Helsmортel (1913).

It seems now well established that the primordia of both leucoplasts and chloroplasts are morphologically alike. If a difference exists, it is not possible at present to distinguish one primordium from another. I am also convinced that these primordia are morphological units of the cell, with the same rank as the nucleus. In addition to these primordia, there is also another organ of the cell to which I have restricted the term chondriosome. These are morphologically distinct from leucoplasts and chloroplasts and are to be regarded also as permanent organs of the cell.

If we are now justified in the view that leucoplasts, chloroplasts, and

chondriosomes are organs of the cell, of the same rank as the nucleus, it follows that these bodies must be transmitted from individual to individual in the form of their primordia and that the chromatin is not the sole carrier of the hereditary characteristics. In my own opinion such a view does not impair one's faith in the doctrine that the nucleus is the chief vehicle of hereditary characteristics, but it does not give the nucleus the monopoly in heredity. There is no sufficiently convincing evidence that the primordia of the plastids and the chondriosomes are carried within the cavity of the nucleus. They must occur in the cytoplasm of both gametes in all plants. Even in such plants as Bryophytes and Pteridophytes there is always enough cytoplasm in the sperm to contain these primordia; so there is no likelihood of their being omitted from any gamete.

The very important question now presents itself: What characteristics are transmitted solely by the nucleus, and what by the primordia of plastids and by chondriosomes? How do these respective characteristics behave in heredity? It is probably admitted on all sides that such characters as are known as Mendelian are carried by the chromatin, but chromatin does not carry chloroplasts, leucoplasts, or chondriosomes. There are many transmissible characteristics that cannot as yet be definitely expressed in any Mendelian ratio. To claim that certain phenomena of fluctuating variability and other numerous characteristics, Mendelian or otherwise, owe their appearance and transmission to the primordia of plastids and chondriosomes may be a daring hypothesis, but, if, as there is good ground to believe, these bodies are permanent organs, there is no escape from some such assumption. If, as some have attempted to show, the pigments grouped under the term anthocyanin are due to a definite granular or rod-shaped body, which is a permanent organ of the cell, are we to conclude that colours, whether behaving in the Mendelian ratio or not, are transmitted by the nucleus? Naturally we are not to think of nucleus, plastids, and chondriosomes as bodies working wholly independently of each other and of the remainder of the living substance, which we call the groundwork of cytoplasm. A nucleus is not known to operate outside of the cytoplasm, nor can these other bodies have any functional existence outside of the cytoplasm. It is, of course, idle to speculate, but nothing is clearer to the biologist to-day than that the living substance has a much greater complexity than was formerly attributed to it. There was a time not very remote when cytologists were somewhat content to believe that dilute solutions of certain acid or alkaline combinations were sufficient to reveal every part of the cell contents visible to high powers of the microscope. At present it is known that while certain parts of the cell are beautifully revealed by the use of certain reagents, other parts are destroyed or rendered unrecognizable by these reagents. Varied and complex phenomena have their basis in varied and complex morphological entities.

## SUMMARY.

1. Leucoplasts and chloroplasts are derived from granular or rod-shaped primordia which are morphologically alike and which are permanent organs of the cell, with the same rank as the nucleus. These primordia multiply by division. Full-grown chloroplasts multiply also by division.

2. In the cells of *Anthoceros*, *Marchantia*, and *Pinus*, and in the tissues of the other higher plants mentioned in the foregoing pages, there are present in the groundwork of the cytoplasm granular and rod-shaped bodies which do not give rise to either chloroplasts or leucoplasts, and to which the name chondriosome is restricted. These chondriosomes multiply by division, and they are permanent organs of the cell.

3. Chondriosomes, as well as the primordia of leucoplasts and chloroplasts, are concerned in the transmission of certain hereditary characteristics.

4. The function of chondriosomes in the cell cannot be definitely formulated at present. They are probably concerned in certain processes of metabolism.

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## EXPLANATION OF PLATE I.

Illustrating Professor Mottier's paper on Chondriosomes and the Primordia of Chloroplasts and Leucoplasts.

All figures were drawn from sections with the aid of the camera lucida.

Fig. 1. A cell from the root-cap of *Pisum sativum*, Marrow Fat variety. In the upper or distal half of the cell are several starch grains (*St.*), in which the plastid appears as a dark, narrow crescent at one side. The dark granules and rods are chondriosomes.  $\times 2,100$ .

Fig. 2. A cell from the root-tip of *Pisum*, showing large rod-shaped primordia of leucoplasts and small, granular, and very delicate rod-shaped chondriosomes. The knob or head at one end of some of the primordia indicates the beginning of a starch inclusion.  $\times 2,100$ .

Fig. 3. An older cell from the central cylinder of the root. The transformation of primordia into leucoplasts is more evident. The starch inclusion in one end of the plastid is circular or oval, giving the leucoplast the form of a pear, club, or hand-mirror. The chondriosomes, as in the preceding figure appear as granules or delicate, slender rods.  $\times 2,100$ .

Fig. 4. Leucoplasts from older cells of the central cylinder of the root of *Pisum*. *a*, chondriosomes; *c*, *d*, and *e*, different forms of leucoplasts. At *c* the oval starch inclusion lies near the centre of the plastid; in *b* three inclusions are present; in *d* the handle of the mirror-shaped plastid remained almost colourless, while the rest retained the stain. This form was rare.  $\times 3,000$ .

Fig. 5. Longitudinal vertical section through the apex of the thallus of *Marchantia polymorpha*, showing one apical cell with adjacent tissue and two young ventral scales. *a* and *b*, mucilage hairs. The chondriosomes in *a* are delicate rods of varying length; some of the rods seem to consist of

## I 14 Mottier.—Chondriosomes and Primordia of Chloroplasts, &c.

rows of rounded granules. In each cell the chloroplasts are the larger round or elliptical bodies, while the chondriosomes are very small granules and delicate, slender, bacteria-like rods.  $\times 1,250$ .

Fig. 6. A cell a little farther behind the region of Fig. 5. *n*, nucleus; chloroplasts appear as rounded or elliptical bodies; the smaller rods and granules are the chondriosomes.  $\times 1,500$ .

Fig. 7. A cell older than that in Fig. 6, from beneath a dorsal air-chamber. The chloroplasts are mature, some showing division stages. The chondriosomes are relatively large.  $\times 1,500$ .

Fig. 8. A tangential view of an older cell, in which the chloroplasts are mainly confined to the thin peripheral layer of cytoplasm; they show a densely-staining shell with a colourless, lenticular inclusion. The chondriosomes are small, slender, homogeneous rods or granules, having, under the dry lens, the appearance of so many bacteria. Some of the rods appear to be composed of granules.  $\times 1,500$ .

Fig. 9. A young rhizoid and a few cells of the lower epidermis. In the rhizoid are seen numerous rods and granules and the conspicuous nucleus. Many of the rods and granules are in process of division. The majority, if not all, of these are chondriosomes. In the epidermal cells, chondriosomes and chloroplasts are seen.  $\times 1,500$ .

Fig. 10. *Pinus Banksiana*. A cell from the cortex of the hypocotyl of a seedling 1 cm. in length. The chloroplasts appear round, oval, or elliptical. In some the inclusion is rounded; in others, lenticular. The chondriosomes are small rods and granules.  $\times 1,500$ .

Fig. 11. Chloroplasts in different stages of maturity, from the same seedling as Fig. 10. *a*, from near base of cotyledon; the inclusions are either round or lenticular in form, two sometimes being present in a chloroplast; *b* and *c*, mature chloroplasts from cortex of hypocotyl. If the chloroplasts are crowded, they become polygonal by mutual pressure, *c*. Some of these mature chloroplasts contain two or more bodies which behave like starch inclusions; others contain only one inclusion as in younger chloroplasts. Between the four chloroplasts at *b* are shown five chondriosomes. At *c* an adult chloroplast is in process of division.  $\times 1,500$ .

Fig. 12. An apical cell of the gametophyte of *Anthoceros laevis*, showing nucleus, chloroplast, and very delicate rod-shaped chondriosomes.  $\times 1,250$ .

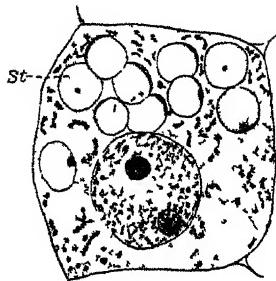
Fig. 13. An apical cell from the root of *Adiantum pedatum*. The relatively small leucoplasts are chiefly lenticular in form; the chondriosomes are rods and granules.  $\times 1,500$ .

Fig. 14. *a*, leucoplasts from the seventh layer of the root-cap of *Adiantum*; each contains two or more lenticular inclusions. *b*, a cell of the fifth layer of the root-cap; the leucoplasts are chiefly lenticular; the chondriosomes are small rods and granules.  $\times 1,500$ .

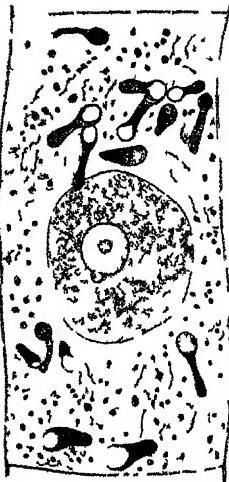
Fig. 15. A cell from the central cylinder a short distance behind the apical cell in the root of *Adiantum*. The long conspicuous rods are primordia of leucoplasts; the smaller rods and granules are chondriosomes.  $\times 1,500$ .

Figs. 16 and 17. Parts of older elongating cells of the central cylinder of the root of *Adiantum*. In 16, at the right, is a cluster of leucoplasts with starch inclusions. The primordia of leucoplasts, 16, 17, that did not develop into plastids are generally very long, thread-like, and flattened. These are undergoing disorganization. The chondriosomes are chiefly rounded granules and are present in countless numbers.  $\times 1,500$ .

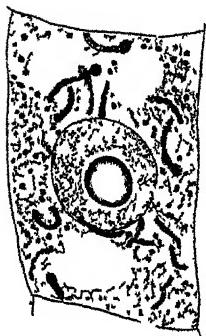




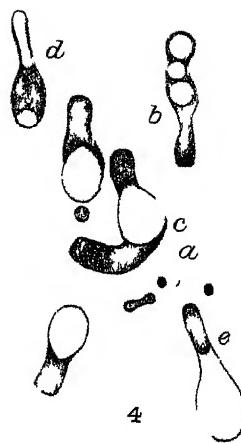
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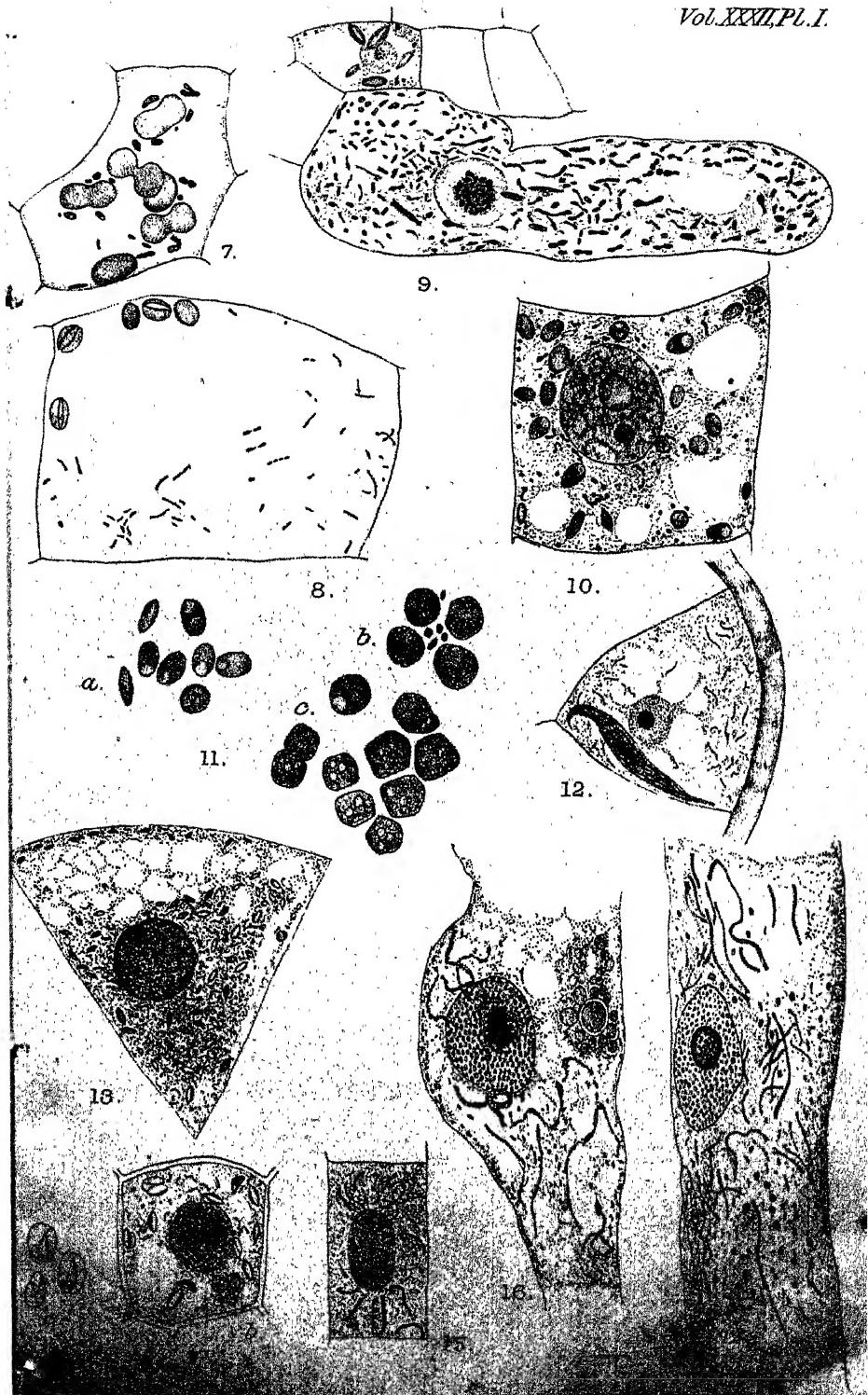


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MOTTIER-CHONDRIOSOMES &c.





# The Morphology and Cytology of the Sexual Organs of *Phytophthora erythroseptica*, Pethyb.

BY

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With Plates II and III.

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THE description of a new species of *Phytophthora*, *P. erythroseptica*, by Pethybridge (27), of such an aberrant type that the author in question considered it necessary to divide the genus as established by de Bary into two,<sup>1</sup> and the subsequent discovery that several other species—*P. Phaseoli* (Pethybridge, 27), *P. infestans* (Pethybridge and Murphy, 80), *P. parasitica* (Dastur, 13), *P. Colocasiae* (Butler and Kulkarni, 7), and probably *P. Arecae* (Pethybridge, 27; Coleman, 11)—had the same type of sexual organs as *P. erythroseptica*, made it desirable to carry out a thorough

<sup>1</sup> Pethybridge (l. c.) named the division of the original genus which has sexual organs of the same type as *P. erythroseptica*, *Phytophthora* because the type species *P. infestans* belongs to it; for the other division, headed by *P. Cactorum*, he established the new genus *Nozemia*. Wilson (Studies in North American Peronosporales. V. A Review of the Genus *Phytophthora*. Mycol., 6, pp. 54-83, Pl. 2, 1914) has advanced reasons to show that the new genus should be called *Phloophthora* (not 'Phleophthora' as given by that author) because the Fungus *Phytophthora Syringae* had first been named by Klebahn from its sexual organs, which were found without mycelium, *Phloophthora Syringae*. This reasoning seems to be based on a misapprehension as to the nature of a pleomorphic life cycle, and is open to grave question. The writer believes that he is following the International Rules (1910), which tacitly exclude the Phycomycetes from other Fungi as not possessing pleomorphic life cycles, and therefore adheres to Pethybridge's nomenclature in this paper.

study of their development and structure. This paper presents such a study of *P. erythroseptica*; and while it would be unsafe to generalize too far in view of the wide variations found in the cytology of the nearly related genus *Albugo*, yet in the absence of further study it may be assumed that they are all alike in cytology and development as they are in mature structure. The work was undertaken in the first place to study the genesis of the sexual organs, but it was found that the nuclear phenomena could be advantageously followed at the same time, and so both studies were carried on side by side. On account of the greater difficulty experienced in the latter branch the work soon assumed an almost purely cytological aspect.

Pethybridge's description of the sexual organs of *P. erythroseptica* may be summarized as follows: The hypha which is about to give rise to an oogonium or oogonial incept, grows up to and pierces the antheridium, grows through it and out at the other side, where it appears as a club-shaped structure and later as a spherical oogonium. He suggested as a possible explanation of this process that perhaps fertilization might take place while the oogonial incept was still within the antheridium. The emptying of the antheridium, the contraction of the protoplasm of the oogonium, and the formation of the oosphere were also described. These observations were all made on living material or on fresh preparations. Pethybridge was unable, owing to pressure of other work, to proceed further with the matter, and he very kindly handed over all his cultures to the writer, who had more time at his disposal. The results which have been obtained with stained sections corroborate Pethybridge's observations in all particulars. It may be stated in general that no theory will fit all the facts but that given by him, and those which give even a very partial explanation are more radical and improbable than the one they seek to replace.

The writer desires to express his indebtedness to Dr. G. H. Pethybridge for furnishing the necessary cultures; to Professor Farmer, under whom the work was begun at the Imperial College of Science and Technology; to Professor Claussen, in whose laboratory it was continued; and finally to Professor Whetzel, who gave the writer every kindness and facility in the Phyto-Pathological Laboratories at Cornell University.

#### TECHNIQUE.

The material used in this study was all derived from pure cultures of the Fungus grown on agar slants and in Petri dishes. At first clear extract-of-oat agar was used, for the preparation of which see Pethybridge and Murphy (30, p. 578). The resulting medium is practically clear, and the stage of development of the sexual organs can be determined under the microscope before fixing. It has no other advantage, however. The Fungus fruits so sparingly on it that a large amount of material has to be sectioned

and an immense number of slides prepared to get even a few of the sexual organs ; besides which, the medium is no better than some other clear media which are more easily prepared, such as potato agar. The main part of the work was done with cultures grown on 'Quaker' oat agar, unfiltered, as used by Pethybridge and Murphy, l. c., p. 580. On this medium *P. erythroseptica* fruits very abundantly, the whole surface being covered with sexual organs. It is difficult to say what advantage there is in using the 'Quaker' oat rather than the unprepared oat, the same formula being employed in both cases, yet there is an advantage (Pethybridge and Murphy, l. c.), and other workers with different Fungi have had the same experience. Careful experiments have been made using equivalent quantities of 'Quaker' oat and whole oat, calculating 20 per cent. of the latter to be husk and using correspondingly larger amounts ; also using the said quantity of whole oat but removing the husk, yet the prepared oat medium always proved the better. The 'Quaker' oat has the further advantage over the whole oat as first recommended by Clinton (10), that it is easier to handle, being finer, and that it contains no husk, which makes sectioning easier.

Preliminary experiments were made to determine the best fixing agents. This was done in the following way : Fine glass capillary tubes were made and cut in lengths a little less than the width of a microscope slide. A drop of the fluid to be tested was placed on the slide between two of the capillary tubes, and to this a portion of mycelium was transferred direct from a young culture. A cover-glass was then laid on the capillary tubes in such a way that it could not crush the mycelium. The slide was placed in a moist chamber to prevent concentration and examined at intervals until the full influence of the fixing agent could be seen. A couple of hours is ample for this. These observations were made almost entirely on conidia, which just then turned up in comparative abundance on a single oat-extract agar plate. It is unusual for *P. erythroseptica* to form conidia on such a medium (Pethybridge, 27 and 28), and later on, when more were needed for the same purpose (for which they are very suitable), resort had to be made to another method to obtain them. If a sterile fly be inoculated and placed in sterile water in a Petri dish an ample crop is produced. The reagent which gave the best results in these tests was Merkel's fluid of the following concentration : Chromic acid, 0.1 per cent. ; platinic chloride, 0.1 per cent. ; and water. It is interesting to note that Claussen had independently arrived at practically the same result in his work. The cytoplasm was precipitated in an exceedingly fine condition, but it was found, when staining came to be done later, that material fixed in chrom-acetic acid gave better nuclear figures with Flemming's triple stain. In the preliminary experiments this fixing agent gave a coarser cytoplasm and often caused contraction. The proper concentration, or more probably the proper pro-

portion of the constituents, was never really found, but the solution recommended by Chamberlain<sup>1</sup> for marine Algae (made up with fresh water however) gave good results. Another solution which was much used was made up of chromic acid, 0·25 per cent., glacial acetic acid, 0·5 per cent., and water, but this was not quite as good as the former. Many other concentrations and solutions were also experimented with, including Schaffner's chrom-acetic acid, Juel's reagent (acetic acid, 2 per cent.; zinc chloride, 2 per cent.; 70 per cent. alcohol, 96 per cent.), and others, but none gave satisfactory results. Flemming's fluid, or any combination containing osmic acid, was not tried owing to its effect on the oil globules in the oospore.

Material was fixed by cutting out pieces of medium which bore a growth of the Fungus and transferring them direct to the fixing reagent. Owing to the opacity of the medium it was impossible to determine the stage of development of the sexual organs, so material had to be fixed serially at given intervals of time to ensure a complete record. A knowledge of how the Fungus fruits helps to this end. If a set plate of oat agar be inoculated in the centre with a moderately large piece of fungus-bearing medium (conidia being practically absent this is the only practicable way) there ensues a rapid growth, visible to the naked eye but at first not aerial. It remains sterile for about three days, and then fruit bodies are formed in the oldest parts of the culture and spread progressively outwards until the limits of the plate are reached. Thus on the fourth day youngest stages may be found just outside the zone where they appeared the day before. This goes on fairly regularly for what may be called the first crop, but young organs continue to arise all over the plate for several days even after the fruiting area has reached the margin. To secure material in regular serial order it is better to discard a plate once some of the medium has been cut out and fixed. The cutting disturbs the regular sequence of events, at least in its immediate neighbourhood. This is seen if a piece of medium be cut out of a young culture in the area where growth is taking place. Sexual organs are formed prematurely on the side of the cut proximal to the point of origin of the growth, and their formation is delayed on the distal side. This may be easily explained if we consider that cutting across the hyphae must cause a concentration of food on the former side, with a consequent tendency towards fruiting, but a diminution of it on the latter, with the opposite result.<sup>2</sup> The usual procedure was to inoculate a number of set plates (twelve to twenty) at the same time, and from the second day

<sup>1</sup> *Methods in Plant Histology*. Chicago, 1905.

<sup>2</sup> Experience gained in several years' work with this Fungus seems to indicate that the stimulus for the formation of resting bodies is not to be found in lack of food or other unfavourable condition, whatever it may be in other forms. It is possible that the comparative lack of asexual organs of reproduction may be an explanation of this phenomenon.

onwards open one of them every twelve hours and fix the part of the medium immediately about the inoculating material. Fixing was done as a rule about midday and midnight, that is, in the latter period of the work. Great difficulty was at first experienced in finding the earliest stages in the penetration of the antheridium by the oogonial incept and the stages of fertilization. It was surmised that both possibly occurred at night : Pethybridge (27) had watched penetration taking place at that time. It was with this in view that the hours for fixing were chosen, and while a greater general activity is found in the material fixed at midnight, the earlier inability to find the missing stages is to be attributed rather to a lack of a sufficient number of sexual organs than to anything else.

Two lots of material were always fixed at the same time—one in Merkel's fluid and one in chrom-acetic acid. Fixation was allowed to continue for twenty-four hours, after which the material was cut into convenient sizes for microtoming. The sexual organs are formed in the upper layers of the medium and they are not oriented in any one particular direction. Consequently sections parallel to the surface or at right angles to it are equally good and show both longitudinal and transverse sections of the organs. Sections cut in the former way, however, are uneven, the lower part of the medium containing no fructifications, while they lie so thick in sections through the surface layers that it is impossible to follow the series of sections through a single oogonium. Some of the best material was spoiled in this way. On the other hand, the fruit bodies are never so abundant that the series cannot be followed in sections perpendicular to the surface.

The material was washed after fixing for from twelve to twenty-four hours in running water and then transferred to 10 per cent. glycerine in an open vessel. Watch-glasses may be used for the purpose if filled to the brim, but they are hardly deep enough, particularly if the material be large in bulk. The vessels should be kept loosely covered to exclude dust. After three or four days at room temperature (or near a radiator if preferred) the watch-glasses or other containers were placed on the top of a paraffin oven for a few hours, or until the liquid had about the same consistency as pure glycerine. The material was then transferred to 95 per cent. or absolute alcohol direct and allowed to remain there about forty-eight hours, the alcohol being changed four times to harden the tissue and to wash out all the glycerine. This step should not be hurried, but it is possible that time might be saved on the preceding one. Up to this stage, provided the proper fixing fluid has been used, one may be certain that even the most delicate tissue will come through in perfect condition, and the process is, in the experience of the writer, much superior to dehydration with grades of alcohol and much less troublesome. If distortion and injury occur at all it is usually in the transfer from absolute

alcohol to xylol. This method of replacing the alcohol was used almost entirely, but accumulating experience has now led to the conclusion that it should be discarded. Successive mixtures of alcohol and xylol containing respectively 25, 50, and 75 per cent. of xylol were employed and the tissue was left about three hours or longer in each. A little eosin was added to the last mixture so as to stain the material red and facilitate embedding and trimming afterwards. Acid alcohol washes it out of the ribbon on the slide in a few seconds. The tissue was finally transferred to pure xylol and allowed to remain there until clear.

The paraffin must be added gradually, a small piece at a time, first at room temperature and, when saturated, at the higher temperature of the oven top. Great care must be taken to prevent crystallization when the temperature drops at night. If the xylol is saturated at room temperature it may be kept overnight in the 25° incubator; or if it be on the oven top, it may be left in safety if covered with an inverted beaker. The whole process is troublesome and unsatisfactory. An attempt was made to devise a simple method of infiltration, and while some success was attained (and indeed the apparatus was used entirely in the latter part of this work with good results), it is not so automatic that it requires no attention or that it does not get out of order occasionally. The method consists in casting thin rods of paraffin in a glass tube, previously coated with glycerine, and inserting them through a hole in the stopper of the vial containing the tissue in xylol. The rod slips down as fast as that part of it which is in the liquid goes into solution. It is probable that the device can be improved. The best material was obtained when left about forty-eight hours, or preferably less, in the paraffin oven. During half that time the xylol was allowed to evaporate before transferring the tissue to pure melted paraffin, but this step does not now seem to be necessary, especially if the xylol be saturated at a fairly high temperature. The material was not embedded in the paraffin to which it was transferred from the xylol-paraffin mixture, but was passed into another bath of pure wax and allowed to remain there a few hours. The same grade of paraffin should be used all through: there is no advantage in saturating the xylol with a wax of lower melting-point and then transferring to a higher. Wax melting at 61° C. was used to a large extent; but it was afterwards found that the 58° C. grade cut equally well, and this was exclusively used in the later work.

An account of how the data relating to the treatment of each lot of material was made and preserved may be useful. Every fixing was given a serial number, and this number, written with a soft pencil on a piece of paper about a centimetre square, was dropped into the fixing fluid with the material and carried with it until they were both embedded in the same cake of paraffin. The same number was given to a slip of paper,

say ten centimetres long by four wide, which was kept permanently with the shell-vial containing the material, and on the slip was noted the length of time every reagent was allowed to act. When the material was embedded the slips were pasted into the notes in proper order. This method has several advantages. One knows at a glance how long the material has been in any particular fluid and the risk of leaving it too long is reduced. The transfer from one reagent to another may be noted as it is being made, and when the slips are preserved they provide an accurate account of the entire treatment which any particular lot of material has received. This is the only way in which mistakes in technique may be detected. Blocks of wood about two inches thick, with holes one and a half inches deep and about one-eighth of an inch larger in diameter than the diameter of the largest shell-vial used, are useful to hold the tissue while it is passing through the various reagents.

Practically all material was cut at five microns thickness. It requires a very sharp knife to cut oospores without tearing them, and there is sometimes difficulty with them dropping out of their oogonia and off the slide. This may be obviated to a considerable extent by using a very dilute solution of gelatine in water, instead of plain water, to straighten the sections out on the slide, and then, when the excess liquid has been drained off and the slide is dry, putting it over a solution of formalin overnight. The very thin film of gelatine is thereby rendered insoluble and anything which was on the slide at the beginning is permanently fixed there. This treatment, along with which it is necessary to use albumen fixative in the usual way, is not generally necessary, however, particularly if the spores be thoroughly infiltrated and the knife sharp.

Flemming's triple stain gave by far the best results in staining, and it was used to the exclusion of all others, although many others were tried. It is particularly noticeable that Heidenhain's iron-haematoxylin gave uniformly bad results. It was tried in three different laboratories at widely separated intervals of time, but in each case was an absolute failure. Very varied periods were used, from a few minutes in each fluid, as recommended by Krüger (22) and others, to twenty-four hours in each, but without success. Altering the concentrations was equally futile. Gentian violet alone or with orange G or other counterstain gave good results. Gram's stain was also used with success. Flemming's triple stain gave the best results with the following formula: Safranin, 1 per cent. solution in 50 per cent. alcohol (made up of equal parts of a 1 per cent. watery and a 1 per cent. alcoholic solution), 1 minute; water, a rinse; gentian violet, 1 per cent. solution in 10 per cent. alcohol, 5 minutes; water, a rinse; orange G, 1 per cent. watery solution, about twenty seconds. The excess stain was wiped off and the slide was dehydrated rapidly in absolute alcohol, followed by clove oil, and differentiated under the

microscope. No attempt was made to differentiate with absolute alcohol: although the process is slower with clove oil the latter extracts the stain better from the cytoplasm, bringing out the orange G and giving a clearer and more pleasing picture. This is especially valuable in the young oogonium, where the cytoplasm has a strong affinity for gentian violet. The clove oil was followed by cedar oil, all the former being carefully removed, and then the slide was allowed to drain and mounted in balsam. This formula works excellently on other Fungi also without modification. Other formulae may be used, and were much used before this one was hit upon, but they do not give as good results.

Many other stains and combinations were tried, particularly in the attempt to stain fertilization tubes. These included light green, eosin, erythrosin, cyanin, acid fuchsin, and methyl green. None of them proved a success, although it is probable that acid fuchsin would have made a good stain for the fertilization tube had there been a little more material at hand to experiment upon.

#### DEVELOPMENT OF THE SEXUAL ORGANS.

##### *Penetration of the antheridium by the oogonial incept.*

In sections prepared from cultures on oat agar about four days old there are no sexual organs of the familiar mature type. Here and there, however, there will be found on the mycelium exceedingly small swellings which are very retentive of safranin and show no details unless that stain be well washed out. Under the oil immersion structures like those shown in Plate II, Figs. 2 to 8 are seen. A more or less irregularly spherical body, the wall of which stains deeply with orange G, is found constantly in close relation to another organ of a club-like shape which has an affinity for safranin and gentian violet. The two bodies are borne on separate hyphae and are clearly of independent origin. The orange body preserves the same staining reaction all through and is evidently the antheridium. The other, which we shall call the oogonial incept, after Pethybridge (27)—assuming for the moment that it is such—when cut in the direction of its long axis, seems to lie either within the antheridium or very closely applied to it.

The evidence that it is within it is grouped under the following heads:

1. Careful focusing shows that parts of the antheridium lie above as well as below the club-shaped oogonial incept when viewed in longitudinal section (Figs. 2 and 3).
2. A section in the plane at right angles to the long axis, or a cross-section, shows one circular body within another (Fig. 4, a). The wall of the outer stains yellow; it is the antheridium. The inner

resembles in all respects the club-shaped body seen in longitudinal section which we have called the oogonial incept.

3. The wall of the antheridium is in favourable sections seen to be invaginated at the point where the oogonial incept seems to enter (Figs. 2, 13, 21). This can be most reasonably explained by assuming that the wall has been actually pushed in by the oogonial incept.
4. A sheath of antheridial origin—staining orange and continuous with the invaginated antheridial wall—encases the lower part of the oogonial incept and extends up it for a greater or less distance (Figs. 13 and 21). This is difficult to explain except on the supposition that the developing oogonial hypha is within the male organ.
5. The appearance seen in Fig. 5 would not be so common, the limits of growth of both organs being exactly the same, if one lay on the other. It is reasonable that such an appearance should be frequently presented if the oogonial incept be engaged in breaking through the wall of the antheridium.
6. In stages like those shown in Figs. 6 and 7, c, the wall of the antheridium is distinctly discontinuous at the points where the oogonial incept is beginning to protrude.
7. In the early stages of the emergence of the oogonium the frayed margin of the ruptured antheridial wall may be seen (Fig. 8).
8. In Fig. 8 the distal margin of the antheridium is seen to be a straight line when looked down upon from above. This is most easily explained by conceding that it has been ruptured here by the emerging oogonium.
9. As the oogonium assumes a spherical form it tends to distend the rim of the antheridium which is in contact with it and turns its margin outwards so that the top of the antheridium resembles the lip of a beaker. Beginnings of this condition are seen in Figs. 9, 10, and 12. This could not take place if the oogonium lay outside the antheridium.
10. In oblique sections the stalk of the oogonium may often be traced passing through the antheridium, entering below at one side and emerging above at the other, or vice versa.
11. If the stalk of the oogonium be not within the antheridium it is not unreasonable to expect to see some indication of that condition occasionally among the very many thousand organs examined. No condition other than that of which Figs. 1, 14, and 16 are the general examples has ever been seen.

If we may assume then that the oogonial incept is within the antheridium the question arises, How does it get there? The oogonial incept

might have arisen first and the antheridium applied itself around it (as happens in many Fungi), the inside walls of the hypha or hyphae which coiled around the female organ then disappearing. This would give the appearance which such an antheridium as we have here presents at maturity. If this did not occur there seems but one other alternative, that the antheridium arose first and the oogonial incept pushed its way into it as has been suggested. To examine the former theory first : The youngest antheridia found (Figs. 2 and 3), in which the oogonial incepts have penetrated only about half-way through, present no indication of having wrapped themselves round the female organ or of having been derived from hyphae the ends or sides of which fused to form a single continuous cavity around another hypha. It is true that a young antheridium previous to being pierced by an oogonium has never been seen. This may be because the stimulus of contact with an oogonial incept is necessary for the formation of the male organ. In *P. infestans* oogonia arise independently of antheridia, for the latter are most frequently missing (Pethybridge and Murphy, 30), and perhaps it is so here too. This was the impression of de Bary and Woronin (5, p. 85). Two antheridia have been found which were not traversed by any hypha. They were well isolated, evidently in relation to no oogonium, but they presented all the appearance of being much older than developmental stages. It is possible that the oogonial incepts which stimulated their formation failed to develop. If this be true, it is easy to see that the finding of an antheridium between the time contact takes place and the stage shown in Fig. 2, or a little earlier, would be by no means an easy matter. Growth takes place at this stage with great rapidity, as Pethybridge (27) has shown. Further, it would be difficult to account for the invagination of the antheridial wall and the *partial* encasing of the stalk of the female organ with a sheath of antheridial origin except on the hypothesis that the oogonial incept does pierce the already formed male organ. In fine, no evidence whatever has come to hand that the antheridium arises by coiling round the young oogonial incept ; and if this be merely negative evidence it is at least derived from a very large number of observations. It is perhaps not a sound argument in the case of such a Fungus to say that it would be unreasonable for the antheridium to encase the female organ at such an early stage as to compel the latter to break through it at the top, when it might more easily arrive at the position the antheridium holds at maturity by encasing the oogonial stalk a little later.

There is no evidence, then, that this hypothesis is sound and it does not explain the observed facts. The only alternative remaining is that the previously existing antheridium is pierced by the developing oogonium. There can be no question, however, how the oogonium emerges. It bursts through the upper wall of the antheridium and forms an oogonium at its summit. It can be seen in the act (Figs. 6, 7, c, and 8). This fact is as

incontrovertible as that at an earlier stage the oogonial incept was entirely within the antheridium. Both rest on observations which seem incapable of misinterpretation. In Fig. 4 are shown two transverse sections of the same antheridium. The oogonial incept is found in one (*a*) within the male organ, while in the other (*b*), which is through the upper part of the antheridium, it is seen that the oogonial incept has not yet penetrated so far. The same condition is shown in Fig. 3 in longitudinal section.

There is strong positive evidence that the oogonial incept in a certain stage of its development is entirely within the antheridium. There is evidence of the same sort to show that it bursts through the upper antheridial wall and emerges freely at the top. And there is every reason to assume—and absolutely nothing to disprove—that the oogonial incept finds its way into the antheridium in the same way as it escapes from it later on, by piercing the lower wall. It is only necessary to follow the stages represented in Figs. 8 to 13 to be convinced that the organ which projects from the top of the antheridium develops into the oogonium. The development has been followed on living material by Pethybridge (27). The structure which we have called the oogonial incept, which pierces the antheridium and grows through it, emerges from its summit and forms the oogonium.

It is convenient to have a word to express this position of the male organ in reference to the female, and we shall use the term 'amphigynous' for it. 'Hypogynous' and 'epigynous' are already in use to describe antheridia, and 'paragynous' might be suggested for such male organs as grow up to the side of the oogonium and pierce it there.

It has been surmised by Pethybridge as a possible explanation of the penetration process that fertilization might take place while the oogonial incept was within the antheridium. This is not so. There is no union of the male and female elements at this time, the wall of the oogonial incept, in which there is no opening or rupture of any sort, keeping them apart. Whatever be the explanation of the penetration of the antheridium, it is not to be found in this. A normal act of fertilization takes place later on.

The oogonia and antheridia are always borne on separate hyphae, as has been stated. The male organ may be terminal but is more often intercalary, and when it arises at, or very near, a point where the mycelium branches it appears to be borne on three hyphae. This is not at all infrequent, as might be expected in a richly branched mycelium. The most frequent condition is that shown in Figs. 7, 4, and 16. The exact time at which it is cut off by a septum, or by septa, from the vegetative hyphae is not clear, and there appears to be considerable irregularity in this respect. Fairly late developmental stages are found in which septa are not yet formed. This possibly is an adaptation to relieve the pressure, which must

be considerable, caused by the penetration of the oogonial incept. At other times there seems to be another way of attaining this end. The antheridium put out a tube of short growth as the pressure caused by the oogonial incept increases, the tube acting as a safety-valve. Growth ceases as soon as the oogonium emerges. Compare in this respect Pethybridge's figures (27). This explains to some extent why so many antheridia seem when mature to be borne on four hyphae.

The earliest stages in penetration strongly resemble the formation of haustoria as described by several authors, and especially by Smith (33) in the Erysipheae. In Fig. 2 the antheridial wall is deeply invaginated, and it can be traced up the side of the developing oogonium until it fades away about where the cytoplasm begins. It is not certain that the wall has yet been actually pierced, although it may have been. If it has, one would expect the tip to be swollen out. The probability is that a very thin and invisible sheath of antheridial origin surrounds it. When this is ruptured the invaginated wall remains surrounding the stalk of the oogonium and acts as a strengthening element, the enclosed part of the stalk being often noticeably constricted, as is shown to some extent in Fig. 21. This is exactly what occurs in the formation of a haustorium (Smith, 33; Gregory, 18), although the goblet-shaped collar described by both these authors is not present.

The oogonial incept arises in every case also from a more or less developed swelling on a hypha which is applied to the antheridium. This may well be compared to the appressoria from which many haustoria develop, as de Bary described (compare also Smith, 33). It might be suggested, however, that this swelling is really homologous with the oogonia of other Oomycetes and that the oogonial incept corresponds to the receptive papilla (see Fig. 2). Against this it may be urged that the appressorium-like swelling shows none of the protoplasmic activities of an oogonium. Traces of nuclear division may sometimes be seen in the hypha leading up to it, but for the most part its nuclei are in the resting condition. There is no trace of zonation or of any other phenomena such as would be expected at the time when the respective papilla appears. A well-developed receptive papilla is formed later on, which seems to rule this argument out.

The comparison with a haustorium, while striking, should not be pushed too far. There is no indication that the antheridium thickens its wall against the advancing hypha, as the host cell does against the haustorium. This has been interpreted as an effort on the part of the host to shut out the invader, and obviously no such action would be expected on the part of the antheridium. It is part of the normal biology of the Fungus. It does seem strange that one part of the organism should react in this way towards another part; and yet the same process is duplicated in

other Fungi. There is no fundamental difference between the penetration process in *P. erythroseptica* and the phenomenon of the well-developed receptive papilla in *Albugo Portulacae* or that of the fertilization tube in any Oomycete. All three are cases of two parts of the same thallus in close contact, one of which pushes into the other: in the one case, pushing well in but not piercing any wall (receptive papilla); in another, piercing one wall but not the opposite one (fertilization tube); in the last, piercing both walls and growing through (oogonial incept). It is probable that all three are due to differences in turgor rendered evident by a protrusion through a part of the wall weakened by enzymic action. At all events there is some evidence to show that receptive papillae and fertilization tubes are only expressions of differential pressures.

#### *Heterothallism.*

The fact that the sexual organs are always borne on separate hyphae,<sup>1</sup> at least so far as they can be traced, led to the idea that perhaps the Fungus was heterothallic (cf. Clinton, 9 and 10). Careful investigation, however, proved this surmise to be incorrect. A brief survey of the experiment will present a few points of interest. Conidia were not available, so single-hypha cultures were made. The medium used was oat-extract agar, which has the double advantage of being clear and of giving a less vigorous growth. Considerable difficulty was experienced in separating the hyphae, and special methods had to be resorted to. The tips of the hyphae when cut off (they had to be very short as a rule) usually died. If a set plate of oat-extract agar be inoculated in the centre and an annular strip of the medium well outside the limits of growth be removed, individual hyphae here and there will cross the clear space and give rise to a good growth in the medium outside. This process was regulated under microscopic control, and if the hyphae were too close superfluous ones were removed with the point of a sterile needle. But this was often unnecessary, the hyphae which crossed the open space being far apart.

Before this simple method was discovered individual hyphae were led across the empty space in very fine sterile glass tubes filled by capillarity with oat-extract agar. The method works occasionally, but it

<sup>1</sup> The condition which Dastur (13) found in *P. parasitica*, which also has amphigynous antheridia, where the oogonium is said to arise as a sort of proliferation of the basal wall of the antheridium, is not present here. The connexion between the stalk of the female organ and a hypha can always be seen in properly stained material, although it is sometimes difficult to make out in fresh preparations when the hyphae are empty, and this may have misled Dastur. The apparent continuity of the stalk of the oogonium and the wall of the antheridium is further heightened by the fact that the base of the male organ is normally invaginated by the frequently much swollen body outside, which has been compared to an appressorium.

cannot be recommended. It is mentioned because, later on, the mycelium in the tube begins to fruit, but the fruits never reach maturity, while mature fruits are abundant at both ends. The abortive oogonia are in part brown, or surrounded by a brown exudation, reminiscent of the abortive oogonia of *P. infestans* (Pethybridge and Murphy, 30); in part they are merely empty and colourless. This is no doubt due to lack of oxygen, at least in the case of *P. erythroseptica*. The fructifications of the latter, even in plate cultures, are all near the surface.

In both these ways several cultures were obtained which were certainly the product of a single hypha. These were planted together on set plates in the way that Blakeslee has made familiar, and the first results obtained tallied so closely with his that it seemed certain that we were dealing with a heterothallic form. Fructifications appeared thickest along fairly regular lines separating the different growths, while later they appeared all over the plate. This might have been due to the intermingling of hyphae of different strains, but it was not. The single-hypha cultures also fruited when grown alone. The explanation is this: While vigorous growth is taking place (up to a certain limit) there is no fruiting; but when growth is suddenly checked in any one place fruit bodies are immediately formed along that line. This happens when two growths meet, the first consequence being a more or less regular line of sexual organs along the junction. Exactly the same effect may be produced by cutting out a piece of medium in the track of growth of a single strain. No doubt the same thing occurs when two species of *Phytophthora* are grown in the same plate, as Clinton (10) did with *P. Phaseoli* and *P. infestans* and claimed to have obtained hybrids. Few details are given, but it is possible that what happened really was that the sudden check experienced by the slow-growing *P. infestans* was just what was needed to stimulate it to normal sexual activity.

#### *Further Growth of the Sexual Organs and Degeneration of Nuclei before Division.*

Both sexual organs in the early stages are filled with dense, almost homogeneous, but slightly granular and vacuolate protoplasm, which stains deeply with gentian violet. The nuclei, particularly in the oogonium, are linear for the most part (Fig. 13). They are in the resting condition, but it is difficult to stain them to show details beyond a nucleolus, which is always present. There is good evidence that they multiply mitotically in the hyphae about to form an oogonium. The nuclei of the antheridium are more nearly spherical. After the young oogonium emerges from the antheridium growth takes place with extreme rapidity. Pethybridge saw one grow to about the stage shown in Fig. 13 in about four hours and

a half. The cytoplasm is stratified in arcs of concentric circles, the centre of which would be somewhere in the stalk (Fig. 13). One has the impression that it welled into the organ in waves or pulses, every wave being flattened out by the succeeding one and made visible by slight differences in density. The nuclei are still linear or bow-shaped, and they lie tangentially to the arcs of cytoplasm. Near the tip the stratification is no longer visible and the nuclei are becoming spherical (Fig. 13). About this stage or a little earlier the oogonium is club-shaped, this being partly due to the fact that the rim of the antheridium acts as a retaining wall (Fig. 12). As the pressure increases this is overcome. The oogonium assumes a spherical shape, distending the rim of the male organ and rounding off its margin until it resembles the lip of a beaker. The normal shape of the antheridium is probably more or less spherical. After the oogonial incept has entered it, and before it emerges, the shape is altered almost to that of a cylinder with rounded ends (Fig. 5), obviously the result of the pressure exerted by the developing oogonium. When the oogonium has reached full size its pressure is exerted in the opposite direction, so that the antheridium now becomes stout and squat (Fig. 13). After the oogonium has emerged the wall of the antheridium thickens appreciably. When very young it may collapse if the cell be plasmolysed, but now it retains its shape under all conditions even though the protoplasm may contract. So far as size and external appearances go the sexual organs are now mature.

After the oogonium reaches its full size there is a progressive alteration in the physical appearance and the staining qualities of the cytoplasm, and this is one of the best means of arriving at the true sequence of events. In the beginning the cytoplasm was densely granular and almost homogeneous, but it now gradually becomes more loosely vacuolate and less deeply staining. The number of nuclei which pass in is 90 to 100, more or less. At the time when the nuclei divide, however, there are only about thirty in the oogonium. It is evident that two-thirds of those originally present must either pass out again or degenerate. It is also possible that some nuclear fusions might occur, but there is no evidence whatever for a thing so unlikely, and the numbers, the average of a good many counts, do not support it. Neither is there any evidence that any nuclei go back into the parent hypha. After the oogonium reaches full size that hypha is practically empty, and it remains so. Besides, the oogonium is separated from it before the full reduction has taken place. On the other hand, all during the period prior to the division of the nuclei there are ample evidences of nuclear degeneration just as it has been described by Claussen (8) in *Saprolegnia monoica* and confirmed by Mücke (26) in *Achlya*. The most conspicuous degeneration figure is a deep-stained undifferentiated sphere, larger than a nucleolus

and smaller than a nucleus, surrounded by a clear halo without a trace of a nuclear membrane (Figs. 15, 16, and 36). This spherical mass becomes smaller and smaller and finally disappears. In the same section many perfect nuclei are to be found. The earliest sign of degeneration is to be observed in certain nuclei which still appear perfect but which stain more diffusely than their neighbours (Fig. 16). The same process goes on simultaneously in the antheridium, but naturally fewer examples are seen (Fig. 15).

This observation, never before recorded in this group, emphasizes the relationship of *Phytophthora* to *Saprolegnia*. It is possible, however, that it may still be found in other members, having been overlooked by all workers on the Saprolegniaceae previous to 1908. Davis had already found it in *Vaucheria* (16), so it is certainly not confined to the Saprolegniaceae alone. There is one other point that must be mentioned in connexion with the resemblance to *Saprolegnia*. Just about the time that the oogonium is fully grown or nearly so there appears, so regularly that it cannot be overlooked, an irregular break in the cytoplasm somewhere between the centre of the oogonium and the stalk, often a little eccentric but never in contact with the wall (Fig. 12). It disappears at a slightly later stage without leaving a trace of its presence. It looks like an artifact, yet its very common and constant appearance, always at the same time too, seems to show that it is not. It recalls strikingly the breaking down of the central cytoplasm in *Saprolegnia*. Whatever it may be, it is not an ordinary case of plasmolysis. The further course of development has nothing more, however, in common with the Saprolegniaceae; it resembles more closely that of the Peronosporales, and especially the Pythiaceae.

While some of the nuclei are degenerating, the oogonium becomes separated from the vegetative hyphae by a thick plug in the base of the stalk, as Pethybridge has described. It is not known how this plug is formed, but it probably originates as a septum, because a septum is found occasionally even in mature organs in place of a plug. The plug and the base of the oogonium stalk surrounding it stain with orange, while the remainder of the oogonium wall takes gentian violet. This is an interesting corroboration of the results obtained by Pethybridge (28) by microchemical methods. It is probably at this time also that the antheridium is cut off by means of septa. These cross-walls usually occur a short distance behind in the hyphae which bear the male organ, and occasionally another cell is cut off behind them, the wall of which thickens and stains in the same way as that of the antheridium.

*Arrangement of the Nuclei during Division.*

In the concluding stages of nuclear degeneration the surviving nuclei are already arranging themselves in the form of a hollow sphere with one in the centre. The term 'zonation' was first used by Stevens (34) to describe the condition in *Albugo Bliti* where the nuclei are arranged about the sharp line of demarcation between the future ooplasm and the periplasm which exists during division. Here all the protoplasm is similar at this time and there is no dividing line until division is complete. According to Stevens's own interpretation of his term in the case of *A. candida* he would not call this the zonation stage. It is best for the sake of uniformity and clearness to use the term exactly in Stevens's sense, restricting it to the time when there is a dividing line between the ooplasm and the periplasm. In the hollow sphere figure (Fig. 17) there is always one nucleus, if not more than one, nearer the centre than the others, which lie in a ring outside. Probably the most correct way to describe the condition would be to say that the nuclei take up positions as far as possible from one another. The result in most cases is one nucleus in the centre and the remainder around the periphery. The protoplasm is now very vacuolate and light-staining, and it is aggregated principally around the nuclei. There is somewhat more of it round the periphery than in the centre, and this becomes more noticeable later on. Otherwise it is uniform all over, within and without the ring of nuclei. There is no denser mass in the centre and there is no trace whatever of a differentiated structure or organ there. All this is in striking contrast to the procedure in *Albugo* and *Peronospora*, but it will be found to resemble *Pythium* very closely. In fact, except that he found no nucleus in the centre, Miyake's (24) figures for *Pythium de Baryanum* might almost as well serve for this Fungus.

As anaphase and telophase come on, the ring of nuclei has moved perceptibly towards the margin (Fig. 18), so that in the normal case the daughter nuclei are almost, but not quite, in contact with the wall. In the final phase the figures seem as a rule to come into the tangential direction. The result is that the daughter nuclei are about equally distant from the margin, but it does not always happen so however. These peripheral nuclei begin to degenerate immediately after division, and the zonation stage now sets in.

The nuclei of the antheridium behave exactly as do those of the oogonium, and events in both organs seem to be quite simultaneous. There is no regular acceleration in division as was found by Krüger (22) in *Peronospora Ficariae*, although it occurs sometimes. It is considerably more difficult to follow the course of events there than in the oogonium, as most previous workers have mentioned. The number of nuclei originally

present is as a rule about eight to ten, but it is very variable. A certain number degenerate: how many it is not easy to say. The number present during mitosis is probably not more than four, so that the proportion of nuclei left to those originally present is about the same as in the oogonium. The cytoplasm never exhibits flow structures nor are the nuclei ever linear. As good mitotic figures may be found in the antheridium as any in the female organ (Fig. 17), and the details of nuclear division are the same for both. The spindles are often oriented in the direction parallel to the stalk of the oogonium, this being necessary on account of the very limited dimensions of the body. Afterwards the same fate befalls the supernumerary nuclei as those of the female organ, nor is it easy to say, until it is seen in the fertilization tube or in the oosphere, which one will serve as the functional male nucleus. It too remains very small until it reaches the oogonium, and it is never clear what fate overtakes the others after division. It is probable that they are reorganized for a very short time after telophase, but that they never reach the stage of resting nuclei again. Their deep-staining, undifferentiated remains persist long after fertilization has taken place and the antheridium is almost empty.

#### *Nuclear Division.*

The nuclei which pass into the sexual organs are very small in size. A general account of their appearance has been given, and the details of the earlier phases need not be gone into further. After those in the oogonium have resumed their spherical shape all the nuclei, in both oogonium and antheridium, behave similarly. This is to be borne in mind even though the oogonium be mentioned oftener than the antheridium in the discussion of nuclear phenomena.

While a relatively large number of nuclei are degenerating in both organs the remaining ones increase proportionately in size. The chromatin masses and the nucleolus stand out clearly, but the presence of a centrosome could not be definitely established at this stage. As soon as the degeneration process is over the survivors have attained their full size and have become arranged in the form of a hollow sphere. They have now entered into prophase. Naturally all the details of division cannot be followed in nuclei so small, and it is not clear how the chromatin is arranged on the linin network or how the spireme thread originates. Beaded chains have been seen, and they are no doubt to be interpreted as the beginnings of the continuous thread. The spireme itself is a conspicuous figure (Figs. 37 and 38). The nucleolus is still large, but not so large in proportion to the size of the nucleus as in the earlier stages. The spireme thread is stout and apparently rather shorter than it sometimes is. In what seem to be the concluding stages, when it is near segmentation, it appears to be much

convoluted. The point where separate chromosomes arise was not seen. When they appear they are small oval or even round bodies, much less in combined volume than the spireme, but still larger than they become later (Figs. 39 and 41). The nucleolus has also begun to diminish in size, and this diminution is continued further during metaphase. The chromosomes are most easily counted now or a little later when they go to the equatorial plate. The number is small and may be stated with considerable certainty to lie between four and six. The formation of the spindle and the marshalling of the chromosomes on the plate have not been followed (Figs. 42 and 43). The activity in this respect of the centrosomes, which, without asters, are afterwards seen at the tips of the spindle, remain problematical. Curved spindles are frequently seen, but whether they represent young stages or not is not always clear.

Once formed the spindle is normally straight and entirely intranuclear (Figs. 42 to 45). At the same time it may remain curved because it is sometimes seen to be of that shape during anaphase. The nucleolus is still present, but it is now reduced to about the size of a chromosome. It may lie in any part of the nucleus, near the plate or by one of the poles. The chromosomes are regularly arranged at the equatorial plate, and they too are now smaller in size and spherical or slightly oval in shape. The spindle is stout at first, but a little later it seems to lengthen out somewhat, the nucleus becoming elongated at the same time (cf. Figs. 42 and 46). Anaphase is now setting in, and the chromosomes begin to travel to the poles one by one—at least they do not go all at once (Figs. 46 and 47). The nucleolus is lost to view and its further fate is unknown. At the same time the nuclear membrane disappears. Telophase is a prominent feature (Fig. 49). The two groups of chromosomes and the spindle between them make up a body of an obtusely oval shape. There is no membrane surrounding it, but the remains of the fibres are still seen stretching across the space between. The daughter nuclei finally result.

The stages following telophase are the most difficult of all to follow, and little can be said about them. The reason for this is that all the nuclei begin to degenerate almost at once, with the exception of one in the centre of each oogonium (Figs. 20, 22, and 23). Of course this holds for at least one nucleus in the antheridium also, but it is even more difficult to follow events there. This places one point at least beyond all doubt—that there is only one division of all the surviving nuclei in both organs. The functional female nucleus appears quite normal (Figs. 22 and 23); it is very small and contains a small nucleolus and a small amount of chromatin. The peripheral nuclei occasionally appear normal also, but they never, in our experience, stain as sharply as the central one. They must begin to break down very soon afterwards, for they are practically always seen in a condition of evident disintegration. This process does not follow quite

the same course as the preliminary degeneration in both organs before division. The nuclei are drawn out and distorted into curious shapes and no membrane can be seen (Fig. 23). The violent movements which are known to accompany the formation of the egg-cell would account for this. It is just what would happen to flabby bodies which had lost their turgor through death or the disappearance of their membrane. When the periplasm has disappeared their remains persist on the outer surface of the oosphere as deep-staining, violet, regular bodies, just like the nuclei which degenerate before division.

As far as one can judge from the figures in nuclei so small, this is not a reducing division. It is unlikely that it should be one, but it will be necessary to examine the division of the spore nucleus to make sure. This does not take place until germination.

#### *The Receptive Papilla or Manocyst.*

After the nuclei have divided there appears a structure protruding from the oogonium into the antheridium in the region between the antheridial collar surrounding the stalk and the rim of the male organ, where the wall of the oogonium alone separates the two bodies (Fig. 21). This is the so-called receptive papilla. No details can ever be made out in a slide stained for nuclear details with Flemming's triple stain, the body staining an intense and uniform red with safranin. For a long time it could not be determined how the structure originated, it being always of mature size when found. This has been the experience of previous workers also. Lately, however, in studying the telophase stage of nuclear division, developmental stages have been found in two cases, in both of which the papilla was only about one-third or less of the size it attains at maturity. Wager (42) has described a granular appearance in the cytoplasm of the oogonium immediately opposite the antheridium just before the structure is due to appear, and Stevens (35) has suggested that this is the sign of enzymic action being exerted on the wall separating the two organs. When the wall is sufficiently weakened the papilla results, not due to true growth but as a swelling, as Stevens has suggested. There is further evidence that this is the true explanation. Up to the time when the oosphere is ripe for fertilization the antheridium is more easily plasmolysed than the oogonium, which indicates that the turgor of the latter is the greater. This disparity is more marked in younger stages than at the time the protrusion takes place, though it is still present then; but it is only when a part of the wall has been weakened that it can manifest itself. This happens in *P. erythroseptica* after the nuclei have divided, and the result is the receptive papilla. It is generally accepted, the writer thinks, that Wager (42), its discoverer, was wrong in considering it homologous

with 'the receptive spot of other ovum cells'; yet the name continues to be used although it has nothing in itself to recommend it. The writer ventures to suggest the term 'manocyst', which at least does away with the implication of a doubtful homology and indicates what is probably the true significance.

The manocyst in *P. erythroseptica* is very large in size, larger even than in *Albugo Portulacae*. It fills up almost one-half of the already diminished volume of the antheridium. When the safranin is well washed out it is seen to be filled with vacuolate cytoplasm which contains no nuclei (Fig. 21). The wall is thin, and now one notices that the wall of the oogonium is considerably thicker than in the earlier stages, and that it shows a double outline. The thickening extends a slight distance around the curve where the manocyst merges into the oogonial wall, and later, when the fertilization tube is pushed out in the opposite direction, the invagination may persist as a slight crater-like swelling still projecting into the antheridium. In spite of the differences due to the special circumstances of the case—the mode of development of the oogonium and the position of the antheridium—there can be no doubt that the manocyst here is homologous with the similar structure in the Peronosporales. Its manner and approximate time of formation and function are evidently the same. True, in this case it is bounded by a wall of oogonial origin only, whereas in all the other cases it is probably bounded by a wall derived from both organs, though Stevens (34) seems to be in some doubt about this. But the point does not seem to be material. Again, it does not appear here at exactly the corresponding time at which it is found in the other Peronosporales. In most of these it precedes the stage of zonation and division (*Albugo Portulacae* is an exception). Here, as has been pointed out, the zonation stage does not appear till after the daughter nuclei are formed, the sequence being the inverse of that in *Albugo*; but zonation and the manocyst appear at relatively the same time in both. For all practical purposes the times correspond. In all cases it comes when the oogonium is in the later stages of maturation prior to fertilization, and it paves the way for that process and makes it possible. Probably its function is to be found in that it preserves a portion of the wall thin and elastic, while the rest is thickened, and so facilitates the elongation of the fertilization tube into the oosphere. Its appearance and staining reaction correspond exactly with those of other manocysts; in fact, it resembles the large cyst-like bodies of the lower Albugos, that of *A. Portulacae* for example, much more than they do the simple protuberances found in *A. candida* and *Peronospora parasitica*. But there can be no doubt that all are strictly homologous.

The manocyst not only appears much later, but persists later than usual in *P. erythroseptica*, and it is a conspicuous object in material of the right

age. The withdrawal is evidently not so rapid as the formation, several instances having been found. This again is contrary to the usual experience, as most authors state, if they mention it at all, that its further fate cannot be ascertained. There is some evidence to show that the wall is not perfectly elastic, so that when the difference in pressure is equalized it does not quite return to its original size or form. Thus one finds slight protuberances still jutting into the antheridium (Fig. 26). Even before this there is evidence of a gradual withdrawal to be found in a very deeply stained mass of cytoplasm lying in the oogonium at the mouth of the manocyst while the latter is still at or near its maximum size. Whether it is entirely withdrawn by contraction, or whether the latter part of the process is one of eversion, is a very fine point and one not easy of solution. Cases which have been seen make it likely that both may occur, depending on the extent to which the wall was stretched, for the size of the body varies somewhat. The further discussion will be taken up under fertilization.

#### *The Oosphere and Fertilization.*

The nucleus which lies near the centre of the oogonium divides at about the same time as all the other nuclei. It is not later as a rule, as found by Krüger (22) in *Albugo candida*; but small and irregular variations found by that author have been noted here too. The two daughter nuclei after division appear at first of about equal size (Fig. 20). They are considerably smaller than the parent nucleus and they stain very much less deeply. Good differentiation is not frequent. Soon afterwards, one of them begins to move away from the centre and at the same time to show signs of degeneration. Its further history is difficult to follow. Sometimes a degenerating nucleus is found at this stage some distance within the margin, but rarely, and it may well be a daughter nucleus from the centre. On the other hand, it may equally well be the daughter of a peripheral nucleus lying a little farther in than usual. It seems probable, however, that the removal from the future oosphere is effected by a wandering out and a dissolution which go on concurrently. However it is accomplished, there is but one functional female nucleus left in the oosphere. It still continues small and stains very faintly, and it often lies in a small irregular mass of slightly thicker cytoplasm which may represent the 'central plasm'. This, however, cannot always be made out. The remaining contents of the oogonium require very careful study to make clear what is happening.

The cytoplasm (Figs. 22 and 23) has reached its maximum attenuation, particularly in the centre. The central nucleus is so small and poor in chromatin that it may even be overlooked unless well stained. Its sister has disappeared, usually without leaving any trace behind it.

On one or two occasions a small spherical deep-staining body has been seen lying beside the surviving nucleus. It may represent the last stage of nuclear degeneration, which would indicate that the process may go on unaccompanied by any extrusion. On the other hand, it is not out of the question that it might be nucleolus, the fate of which, in the last stages of division, it has not been possible to trace. The peripheral daughter nuclei lie on a regular line very near the periphery. In an occasional case perfect nuclear structure may be made out in them, but nearly always they are evidently in a state of rapid disintegration. The first sign of this is again a diffuse staining. They become drawn out tangentially, staining with safranin, but not sharply. In certain areas they form an almost continuous line separating the cytoplasm which lies inside from that outside. In other places there are small granules, with the same staining reactions as the disintegrating nuclei but of uncertain origin, which give this line a further appearance of continuity. Close examination of favourable sections shows moreover that there actually is a line—a membrane—forming a boundary between the two areas of cytoplasm independent of the nuclei (Figs. 22 and 23). The latter appear to be drawn out at the ends so as to contribute to the formation of the membrane, but if they contribute to it in any way it is probable that it is merely laid down on their inner surfaces where they cleave the protoplasm. Close study shows too that there is a differentiation in structure and staining reaction between the cytoplasm inside and outside. The inner is extremely thin and hyaline, staining practically not at all; the outer takes the orange slightly and seems of quite uniform structure, while the other is vacuolar. This is the zonation stage: the oosphere is now differentiated from the very scanty periplasm.

Slides showing this condition have long been known, but their meaning and importance were misunderstood until the work was nearly finished (26). This was because the writer could not make up his mind that there was an actual membrane along the line of peripheral nuclei separating the cytoplasm inside from that outside. The figure was interpreted as showing the wandering out of the nuclei and the bodily contraction of all the cytoplasm to form the oosphere. In such a case there would be no periplasm. The next stage that one finds certainly shows an oosphere (or oospore, for it is usually fertilized by this time, but still with a very thin wall) which contains all the protoplasm of the oogonium (Fig. 27). It was natural to assume that it had been formed by the simultaneous contraction of all the contents into one mass. That is not so however. There is a periplasm differentiated for a short time and separated from the oosphere by a plasma membrane. Its rapid disappearance is no doubt to be explained by assuming that it has been absorbed by the oosphere, leaving no trace behind but the nuclei which lay on its inner margin. It

is worthy of note that the nuclei are not distributed throughout the periplasm as they are in *Peronospora*. Such an absorption has been described by many authors in the case of *Pythium* and to a less extent in that of *Phytophthora*. The matter is dealt with more fully in the discussion at the end of the paper.

At the time when the periplasm is disappearing the fertilization tube<sup>1</sup> begins to be pushed in (Figs. 24, 25 a, and 26). The tube is not produced in this Fungus until the oosphere is almost ready for fertilization; in striking contrast to the procedure in *Albugo*, where it may be formed while the nuclei are still in prophase, and in contrast to that in most other Peronosporales to a less extent. It is another link in the long chain connecting *Phytophthora* and *Pythium* however (Miyake, 24). It seems probable that it is actually pushed in. The turgor of the oogonium has now fallen so low that it is exceptional to find one which is not contracted a little somewhere, while the antheridium never contracts at this time. The pushing in of the tube and the act of fertilization take place while the periplasm is being absorbed by the oosphere. It would be impossible to say that one regularly precedes or succeeds the other, which is also a point of resemblance to *Pythium* and to *Nozemia Fagi* (Hartig, 19). Consequent on its functioning very soon after formation the tube is exceedingly difficult to find while still containing a nucleus (compare Miyake, 24). This is aggravated by its small size—as a rule it does not penetrate farther than is shown in Fig. 25, a, though cases were observed where it reached nearly to the centre. Sometimes, however, it is even shorter than this, as shown in Fig. 26, and as the position of the male nucleus testifies, which often lies in contact with the spore wall near the antheridium. Further, it stains very lightly and all attempts to stain it more clearly were failures. As a consequence only a comparatively few examples were found although a large amount of time was spent examining material of about the right age. It was considered, however, that the cases already found demonstrated the point clearly enough and that further expenditure of time would not be justified. The contracted and very hyaline remains of the tube are quite commonly found after fertilization is accomplished, lying as a rule in the now empty oogonium or running out towards the developing oospore (Fig. 28). It was in this way that the structure was first found. The discarded tube proves one thing which is not always clear otherwise (see Fig. 26)—that there actually is a tube and not merely an opening between the two organs. The tube-like nature and the opening to the antheridium can often be made out in the empty condition.

<sup>1</sup> It might be suggested that this structure is not really homologous with the fertilization tube of other Oomycetes since it is part of the oogonium. It is, however, quite comparable to the tube in species of *Pythium* with 'stalk' antheridia, which is always called by the same name. Further, it has never been shown in the case of paragynous antheridia whether one organ or both form the tube, and if one, which.

The tube contains a single nucleus near the tip, and after it a large part of the cytoplasm of the antheridium passes in. Fig. 26 shows this actually in the act. This is in agreement with Hartig's (19) account of *Nozemia Fagi*, and in agreement with most other workers on *Pythium* and on *Phytophthora*, but in direct contrast to de Bary's (2) rendering of the procedure in his account of *Phytophthora*. All authors agree that the antheridia of *Pythium* are left almost empty after fertilization, and this is the impression one gets here too from a study of both living and fixed material. The remaining nuclei and a small amount of cytoplasm are left behind. Two very small deeply staining bodies, possibly the remains of two nuclei, were once found in the base of a tube which had already functioned, but this is quite exceptional. The tube having penetrated a short distance into the oosphere delivers its nucleus and cytoplasm, and is then in all probability cast out. The male nucleus is small and shows little structure beyond a large nucleolus. In one case at least it was found somewhat pointed at one end (Fig. 25, a). Meanwhile the female nucleus is increasing in size and staining power. As a rule it remains small and stains lightly until after fertilization, but these events are evidently not entirely interdependent, because now and then one sees cases in which the fertilization tube is formed rather early, while the periplasm is still fully present; again, fertilization may be delayed till the oosphere nucleus has gained in size and chromatin and the contraction of the periplasm is more marked. This is usual in the whole group.

It seems likely that the oosphere contracts somewhat during or after fertilization. In many cases the periplasm is so exceedingly scanty that the degenerating nuclei are only just perceptibly removed from the oogonium wall. When the periplasm has disappeared the young oospore is never so large. The cytoplasm of the oosphere also becomes markedly denser at the same time. However little or much this contraction may be, the limits are soon reached, and the oosphere, now containing one male and one female nucleus, surrounds itself with a thin wall, which Stevens has termed the primitive wall (Fig. 27).

Outside this there is nothing at this time in the way of periplasm except the remains of the nuclei which still stain deeply. Except these and an isolated fragment or granule perhaps, the space surrounding the spore is empty. The greater aggregation of periplasm about the fertilization tube which de Bary and Woronin (5) found in *P. omnivora*, in which they found little or none elsewhere, has not been seen. Neither are the trabeculae, described by Pethybridge (27) for this Fungus and for *P. Arecae*, by Coleman (11), very evident in the writer's slides. They must stain very faintly, and it is possible that they do not persist very long.

There seems to be little point in dwelling on teratological cases unless they appear to possess a phylogenetic or other significance. Curved ferti-

lization tubes of abnormal length were mentioned, however, in a preliminary paper on this Fungus (26), and the point deserves reference on that account. Such fertilization tubes do occur, but they are quite abnormal. They appear to function in the normal manner however. In one case one of them was found still embedded in the oosphere and a nucleus lay by its tip. The connexion of the tube with the antheridium could not be traced. In another case this connexion of a similar tube with its antheridium was placed beyond reasonable doubt. Further, it occasionally happens that parts of such long, curved tubes are retained within the oospore and there coated with a deposit similar in nature to the endospore. This phenomenon has been described by many workers on the cytology of the Oomycetes.

Another abnormality which may be of more significance will be mentioned. In this case the antheridial sheath surrounding the stalk of the oogonium was developed to such an unusual extent that it entirely covered the portion of the stalk within the male organ. Instead of forcing the fertilization tube in through the double wall, the antheridium had apparently grown out a short distance at one side so as to come into contact with the spherical portion of the oogonium, where of course the wall consisted of a single layer only. The outgrowth was appreciably thinner walled than the remainder of the antheridium, which seems to show that it was of later origin. Fertilization had already taken place, but the fertilization tube could still be traced running from the outgrowth into the oosphere. Only one such case was seen.

#### *The Oospore.*

As the oosphere contracts a gradual increase in the density and staining character of the cytoplasm results (Fig. 28). When fertilization is accomplished the cytoplasm begins to take on the appearance common more or less to all oospores in the group. It stains more brown than orange and has a tendency to accumulate in clumps of a granular nature. The endospore is gradually being laid down meanwhile, and while this is going on the inner boundary of the wall is never sharp. The endospore consists of two layers, named by Stevens (34) in the case of *Albugo Bliti* the primary and secondary endospore respectively. They are made evident by their staining reactions; the former, which is much the thinner, appearing somewhat hyaline, the latter staining more deeply with orange G. The mature spore wall (Fig. 35) thus consists of three layers: (1) the primitive wall, represented by a single stout line, which does not take the orange. This is the first to be laid down and is no doubt the last which remains on germination, as Pethybridge (28) has shown. (2) The primary endospore, a thin hyaline layer taking orange slightly. This is probably of the nature of a reserve

material, as is certainly (3), the secondary endospore, a thick layer which stains brown with orange G. Compare Pethybridge (l.c.), where he shows that it is dissolved prior to germination.

There is no deposit of the nature of an exospore laid down outside the primitive wall. This, correlated with the absence of periplasm after fertilization, explains the smoothness of the spore in the species, as indeed probably in the whole genus and in *Pythium* (to a less extent) and *Saprolegnia*. So far as the writer knows the oospores are always irregularly marked in the Albuginaceae and Peronosporaceae, that is, in families in which the periplasm is known to persist. The mature oospores are spherical, smooth-walled bodies, the wall being about two microns or a little more in thickness. If they happen to be formed in a confined space they adapt themselves to the form of their surroundings. It not infrequently happens that the oogonium, after it has grown through the antheridium and become spherical, puts forth a club-shaped outgrowth. The nuclei which pass into this divide and go into zonation normally, the oosphere and oospore which result retaining the abnormal shape of the organ which contains them.

All during the formation of the spore wall the two sexual nuclei remain apart. If the male was originally in contact with the wall it remains there and may be seen even flattened out against it (Fig. 31). Both nuclei grow considerably in size. They now show a distinct nucleolus and chromatin masses, and rarely a trace of linin network. When the wall is nearly mature they begin to approach and finally come into contact, the fusion taking place in a leisurely fashion judging by the number of cases found (Figs. 32, 33, and 34). The part of the membrane separating the two nuclei disappears and the contents begin to mingle. Eight-shaped figures representing this condition are not very uncommon, and in the next stage is found a rather elongate nucleus of a large size without any constriction. Finally an oval or spherical resting nucleus results. The last stages in particular are difficult to stain well on account of the accumulation of coarse oil globules in the cytoplasm and around the nucleus. Early in the formation of the spore one sees these oil drops being formed, very small at first and then increasing in size (one can see them coalescing) and number. At first they seem to be of a solid substance, for they persist in the fixed material. Later these disappear, and instead one finds occupying much more than the greater part of the spore a large empty space, which contained oil in the living condition. The cytoplasm becomes more and more gathered into clumps with increasing spaces between. Finally it occupies a relatively small area of the spore; most of it is distributed around the wall in a thin layer, but there are a few aggregations of larger size, in one of which the nucleus lies (Fig. 35). The oospore rests in the uninucleate condition, but its further cytology has not yet been worked out.

## GENERAL CONSIDERATIONS.

The results of cytological investigations on the Oomycetes have been so much discussed that it is not proposed here to do more than present a short summary of the more recent work. It would serve no useful purpose to recall now forgotten controversies that waged round the question of the presence or absence of nuclei or of fertilization in the Saprolegniaceae. Even the pioneer work of Wager up to 1900 may be passed over because all the forms he worked with have since been reinvestigated by himself or by others. The same may be said of Trow's earlier researches on the Saprolegniaceae, and of Berlese's work on the Peronosporaceae.

The modern period for us begins with Stevens's paper on *Albugo Bliti* in 1899 (34). Further papers by the same author (35 and 37) deal with *A. Portulacae*, *A. Tragopogonis*, *A. candida* and *A. Ipomoeae-Panduranae*. Davis (14) investigated *A. candida* again and Krüger (22) reinvestigated the same species in 1910. Finally Ruhland (32) worked on *A. Lepigoni*, which completes the list of work done on that genus.

Turning to the genus *Peronospora*, the first work of which we shall take account is Wager's (42) on *P. parasitica*. Ruhland (32) reported work on *P. Alsinearum*, *P. affinis*, and *P. Violae*; and Krüger (22) investigated *P. Ficariae*.

Two species of the genus *Plasmopara* have been examined: *P. alpina* by Rosenberg (31), and *P. densa* by Ruhland (32).

*Sclerospora* has been investigated by two authors also: Stevens (36) worked on an unnamed species, and Ruhland (32) investigated *S. graminicola*.

Trow (40) and Miyake (24), both in 1901, examined *Pythium*, the former *P. ultimum*, and the latter *P. de Baryanum*.

The work done on the Saprolegniales is given only passing reference and will be just enumerated here. Trow (39) worked on *Achlya americana*, and later (41) on *A. polyandra*, Hildebrand, and *A. de Baryana*, Humphrey. Mücke in 1908 (25) reinvestigated *A. de Baryana*, Humphrey, under the name of *A. polyandra*, de Bary. Davis (15) worked on *Saprolegnia mixta* in 1903, choosing an apogamous form; and Claussen (8) investigated *S. monoica*. The list is concluded with the work of King (20, 21) on *Araiopora pulchra*, one of the Leptomitaceae.

Even in the field of cytology it would be difficult to parallel the diversity of findings and of conclusions of these authors. Thus in the case of *Albugo candida*, which has been examined three times since 1900, Davis (14) found that there was one division in the oogonium, that the ooplasm was for a time nucleus-free, and that there was an organized coenocentrum. Stevens (35) found two divisions in the oosphere, which was never nucleus-free and contained a coenocentrum with inclusions and attractive functions.

Krüger (22) found only one division in the whole oogonium, the future oosphere never nucleus-free, and no organized coenocentrum. The differences between Stevens and Ruhland on the genus *Sclerospora*, possibly on the same species, are no less marked. Similarly Trow (40) and Miyake (24), working on two species of *Pythium* in 1901, arrive at totally different conclusions on the arrangement of the oogonial nuclei at zonation, on the nature and fate of the periplasm, and on the presence of an exospore. The first is not perhaps of prime importance, as Krüger (22) has suggested. Whether or not there is one nucleus, or more than one, or none, in the centre of the oogonium during mitosis is more or less an accident and may well vary in the same species. Even when a nucleus does appear to lie in the central region it is not always in the exact geometrical centre, and the others are not all equally distant from that point. This seems to be a case where individual variation and the personal element are largely responsible for divergent conclusions. The other two points are of more importance—the presence of periplasm and of an exospore. Trow shows a zonation figure with dense periplasm and clearer ooplasm such as one finds in *Peronospora*, and later shows the oosphere entirely separated from the periplasm by an empty space. The periplasm is formed much in the same way as in *Phytophthora*, by the aggregation of the denser cytoplasm round the periphery, but it is larger in amount. He suggests that it is absorbed by the young spore, which is likely, seeing that it disappears without leaving any trace, and no exospore is formed. Miyake evidently had excellent material, and his drawings emphasize the similarity of behaviour in *Phytophthora* and in *Pythium*. Zonation is not nearly so obvious as Trow figured it; the periplasm is very small in amount, and at the time of fertilization it is almost gone. It seems clear that the author was mistaken in supposing that it functioned in forming an exospore, because none is present. What Miyake calls the exospore is possibly the primitive wall. Trow was probably right in this respect.

Apart from this discrepancy the similarity of the cytological activities of *Phytophthora* and *Pythium* (so far as the two species examined are typical) is remarkable, so much so that Miyake's figures might almost as well serve for this Fungus. In both all the cytoplasm of the oogonium remains undifferentiated till well after division. The fertilization tube is formed only just before it functions and it is then short. The oosphere is formed in the same way, by the ultimate absorption of all the cytoplasm, leaving the superfluous nuclei outside. All this is in striking contrast to the procedure in *Albugo* and *Peronospora*, and it emphasizes the very close relationship of *Phytophthora* to the Pythiaceae. At the same time there appear to be characters connecting it with the Saprolegniaceae. The degeneration of nuclei in the oogonium of *Phytophthora* prior to division indicates unmistakably a close relation with *Saprolegnia* and

*Achlya* at least, where the same thing has been found to occur. This assumes that the account of all previous workers on the Peronosporales, where it has not been found, to be correct. It would not be surprising to find that it took place in *Pythium* at least.

It is less easy to analyse from the standpoint of their respective nuclear behaviours the relationship of *Phytophthora* to the remaining Peronosporales. This is partly due to the obscure relation of these to one another and partly to discrepancies in their various cytological histories. In the first place, it is evident that the Albuginaceae stand apart. This is supported on morphological and parasitological evidence. Whether they lead up through *A. candida* and *A. Lepigoni* to *Peronospora* is another question. It is easy to recognize an oogonium of either genus, from its cytology alone; they belong to different groups. In *Albugo* the ooplasm is very dense and is separated from the periplasm by a very sharp line of demarcation, along which the dividing nuclei lie. *Peronospora*, on the other hand, has its periplasm denser than the ooplasm; the dividing line is not so sharp; and the nuclei do not lie so regularly on it. Further, in the lower species of *Albugo*, those with multinucleate ooplasm, there is always a second division of these nuclei, according to Stevens (34, 35). The same author states that the ooplasm nucleus of *A. candida* divides a second time also (35), but in this he is at variance with Davis (14) and Krüger (22), the latest worker on the group. Indeed, leaving the above-mentioned lower species of *Albugo* out of account, the weight of evidence is gradually coming round towards establishing only a single division in each of the sexual organs. Some of the earlier workers were obsessed with the idea that there must be a reduction division at that point, and it is wonderful what can be seen under such circumstances. It has now been shown on fairly good evidence in the case of *Saprolegnia* (Claussen, 8) and *Albugo* (Krüger, 22) that reduction takes place in the first division of the fusion nucleus, and this no doubt holds true for the whole group. The second division is intelligible in *Albugo Bliti*, *A. Portulacae*, and *A. Tragopogonis*, but hardly in a form like *A. candida*. While, if it may be accepted that the latter has only one division, it certainly shows signs of a possible connexion with *Peronospora*.

All workers on *Peronospora* agree that there is only one division in both sexual organs, and that the cytoplasm about the same time is sharply divided into two zones—an inner vacuolar and an outer denser area, the periplasm. On all other essential points the authors are in unanimous agreement also, with the exception that Krüger (22) shows that the structure described by Wager (42) and Ruhland (32) as a coenocentrum is merely the irregular little mass of cytoplasm that is sometimes found in the centre (*Zentralplasma*). Krüger describes too, without figures, a repeated division of the oospore nucleus while the wall of the latter

is being laid down. This is in opposition to the findings of all other authors and it is fairly certain that it does not occur: it is possible he mistook fusion stages in the spore for divisions, neither of which is figured. The continuation of the uninucleate condition of the oospore throughout the resting period might be construed as an extension of the tendency found in the genus *Albugo*. *A. Portulacae* and *A. Bliti* are multinucleate throughout. *A. Tragopogonis* and *A. candida* have oospores which are uninucleate for a short time only. The oospore in the genus *Peronospora* is uninucleate all through the resting period.

This fact, coupled with a certain degree of resemblance during zonation, appears to present some cytological evidence of relationship between *Albugo* and *Peronospora*. There is other evidence derived from the same source, however, which tends to emphasize the relationship of *Peronospora* to the other genera of the Peronosporales. This is of course the general view based on morphology alone, and for that reason it is doubly likely to be the right one. These genera, *Pythium*, *Phytophthora*, *Sclerospora*, and *Plasmopara*, have not been investigated very fully, but enough has been done to show that they are very closely related. One point in particular of considerable importance has been well established but not sufficiently appreciated. That consists of the marked difference between them and the genus *Albugo* in the appearance of the cytoplasm in the oogonium, the late appearance of zonation, and the small amount of periplasm which they all possess.

Stevens (36), who worked on an unnamed species of *Sclerospora*, and Ruhland (32), who investigated *S. graminicola*, are in considerable disagreement on several points, but they are in fair agreement on the question which more nearly concerns us here. Both refer to the lack of differentiation of ooplasm and periplasm in the early stages of nuclear division. Stevens found that zonation set in later, and he figures an undoubted periplasm both after division and when the spore is maturing, which is, however, less than in *Peronospora* or *Albugo*. Ruhland, some of whose drawings of the formation of the oosphere and the extrusion of the superfluous nuclei in the oogonium rather resemble those of *Phytophthora*, suggests that the periplasm is absorbed to feed the spore, since the exospore is so small; and in no case does he show it after fertilization is accomplished.

Much the same thing is found in the genus *Plasmopara*, which is evidently very near *Sclerospora*. Ruhland (l.c.), who treats of both in the same paper, makes use of practically the same words to describe them: there is no differentiation of the oogonal cytoplasm during division; the periplasm is scanty, and he suggests that it is used up to form the endospore. There is very little shown in the figures. Rosenberg's (31) account and drawings on the same point in *Plasmopara* are quite con-

firmatory. He shows periplasmic nuclei in mitosis (?) after the delimitation of the oosphere which are practically devoid of all cytoplasm. There can be little doubt that Rosenberg was mistaken in supposing that the oogonial nuclei divide a second time. It is common to find elongated and otherwise distorted nuclei at this stage in *P. erythroseptica* also, which at first sight simulate division stages, but which certainly are not. The author has also seen spindles in poor material (particularly in a lot of slides which were stained with Gram's stain) which exactly resemble the spindles Rosenberg (and Stevens also) considers typical of the second division, yet it can be stated positively that there is only one division in this Fungus. But whether there be one or more mitoses in *Plasmopara* one can see that after division the superfluous nuclei are extruded and all the protoplasm ultimately finds its way into the oosphere, just as it does in *Phytophthora*.

It is clear that *Pythium*, *Phytophthora*, *Sclerospora*, and *Plasmopara* are closely related in the organization of their ooplasm and periplasm. They agree also, both among themselves and with *Peronospora*, in having no coenocentrum comparable to the granular mass in *Albugo candida*, in the delayed fusion of the sexual nuclei, and in division of the fusion nucleus being so retarded that they all (probably) rest in a uninucleate condition. Morphological and biological evidence suggests strongly that these genera including *Peronospora*, form a natural group, and it would require strong cytological evidence to prove the contrary. *Peronospora* must be derived from a form like *Plasmopara* or *Albugo*. Morphological considerations undoubtedly point to *Plasmopara*; the cytological seem to point in both directions. If the classification be based mainly on cytology there seems to be a fairly close relationship between *Albugo candida* and *Peronospora*, but if particular emphasis be laid on morphology the difference between them is wide. On the other hand, the morphological and biological evidence that *Pythium* and *Peronospora* are closely connected is so perfect that the conflicting deductions arrived at from cytological study must be interpreted as supporting the biological and other evidence that connects *Peronospora* with the other genera which at present make up the Peronosporaceae. The consequence of this is that the Albuginaceae are placed apart from all the other forms, and that the remaining genera of the Peronosporales fit into a gradually ascending series in somewhat the following order: *Pythium*, *Phytophthora*, *Sclerospora*, *Plasmopara*, and *Peronospora*. Those forms only are included the cytology of which has been worked out. It is evident from a consideration of this series that the periplasm is an increasing structure (in this particular case at all events), that is, if one takes a full view of the series and compares *Pythium* or *Phytophthora* with *Peronospora*. It is less easy to be sure from a consideration of other investigators' accounts and figures, particularly

when they paid little attention to the matter, that there is a gradual increase from *Phytophthora* to *Plasmopara*. It seems to be so, but a careful comparative study would be necessary before passing final judgement. In the case of *Pythium* and *Phytophthora* there can be no doubt that the periplasm is very scanty and ephemeral. This is a point not generally appreciated, although it has been noticed by the earlier workers on both groups and by others since. De Bary (3, p. 134) in describing the genus *Pythium* says, for example: 'The space between the oosphere and the wall of the oogonium continues to be filled with a slightly granular, hyaline protoplasm, the periplasm, which may easily be overlooked.' Again he says (l. c., p. 135): 'In *Pythium* and *Phytophthora* the periplasm cannot be seen to take part in the maturing of the spore; it surrounds it in the form of an inconspicuous, sparingly granular mass.' Referring to *Peronospora* he says (l. c.): 'The periplasm in the oogonium is much denser and more copious.' Marshall Ward (43) describes and figures a very coarsely granular cytoplasm in *Pythium de Baryanum* and says that it disappears in two and a half hours after fertilization. He, as well as de Bary, of course, worked on living material.

Cornu saw no periplasm in *Pythium*, and Hartig (19) none in *Nozemia Fagi*. De Bary (2) in his description of what he asserted to be the same Fungus (*P. omnivora*) says that it does seem at first sight that the peripheral part of the oogonium is 'von wasserheller Flüssigkeit erfüllt. Bei näherer Untersuchung erkennt man jedoch ringsum eine stellenweise ungleiche, manchmal selbst feinkörnige, wolkige Trübung.' No episore is formed; and then he says: 'Zuletzt zerfällt die ganze Masse unregelmässig in Klümpchen oder Tropfen, welche schliesslich kaum mehr zu erkennen sind.' Fischer (17) says: '*Pythium* hat nur sehr wenig, vielleicht bei einigen Arten gar kein, Periplasma.' Such examples might be multiplied.

It is possibly true, as Fischer says, that in some species of *Pythium* there is no periplasm, even if there be some in others. Such a variation within the limits of a genus need not surprise any one, particularly a 'transition' genus like *Pythium*. *Monoblepharis* presents the same phenomenon. Thaxter (38) has described a species in which the whole of the protoplasm of the oogonium is not included in the oosphere, a portion of it protruding from the aperture to attract the sperms. In other species investigated by Lagerheim (23) the material which attracts the male cells is not visible, all the protoplasm being used up in the oosphere. The throwing out of bodies from the rounding-off eggs of species of *Saprolegnia* described by de Bary may be taken as indicating the beginning of a periplasm, and Butler (6) says that de Bary regarded them as homologous with such. The occasional finding of an exospore-like structure in *Pythiopsis* is to be regarded in the same light, as its discoverer (de Bary, 4) held. However,

the whole matter calls for further investigation, and there is no richer field for cytological research than *Pythium* and *Pythium*-like forms.

From the knowledge at our disposal it is easy to see that the Pythiaceae and *Phytophthora* have on the whole a generalized type of sexual reproduction, one which leads to the more specialized type of the Peronosporaceae. The organization of ooplasm and periplasm prior to fertilization is developing, but it is not established so clearly as in *Peronospora*. On the other hand, the likeness of some species of *Pythium* in the mode of their sexual reproduction to *Myzocytium* and *Lagenidium*, for instance, as has often been pointed out, is still more striking. *Pythium* is not at the bottom of this line of development, as de Bary held, but rather one at least, perhaps two or more, steps up. Morphologically there is little distinction between the intercalary oogonia and antheridia of certain species of *Pythium* described by de Bary and Woronin (5), and especially between those of *P. rostratum*, described by Butler (6), and the Ancylistales just mentioned. Cytologically the difference is not so great as is often imagined. In the Ancylistales the whole of the protoplasm of the male cell unites with the whole of the protoplasm of the adjoining female organ by means of a short fertilization tube before the contents of the oogonium have been withdrawn from the wall or organized into an oosphere (12, 44). It is evident that *Pythium* and *Phytophthora* are intermediate between this condition and that found in the Peronosporaceae. The linking up of the Ancylistales with this series seems to make it a logical necessity to derive the higher Oomycetes from the Chytridiales, and through them from forms like *Protococcus*. This line of descent has been argued with considerable force by Atkinson (1) recently from other premisses, and it seems that the points brought forward here add some degree of strength to it. The author realizes the many aspects of the problem, some of which are entirely neglected here—for example, the significance of the Monoblepharidales and the possible rôle that parasitism has played. It must be confessed that to the writer at least it came as a surprise to have to admit that according to the theory outlined above the periplasm must be an increasing structure. If on the other hand we hold that the lower Oomycetes have resulted through a life of parasitism, then we arrive at the conclusion that the periplasm is decreasing. This view seems to have lost much ground, based as it was on a false analogy of plants to animals. If parasitism is really responsible for the degeneration of the Chytridiales from the Peronosporales or the Saprolegniales (de Bary, 3 and 5), did the Fungi which are now parasitic on the forms from which they are said to have arisen begin by parasitizing their own close kin? Such a thing seems in the highest degree unlikely. If this has been the course of events they must have become parasitic on another form first and then transferred their attentions to other descendants from their own direct ancestors.

Such a phylogeny as has been indicated leaves out of account the Albuginaceae also. It is likely that they must be derived on a parallel line from the Leptomitaceae, which are similar in other ways besides being richly provided with periplasm, but which, in the species *Araiopora pulchra* (King, 20 and 21) at all events, have a uninucleate oosphere. There is no reason, however, why *Albugo candida* should not be the lowest member of a series which culminates in *A. Portulacae*. Stevens (35) has given reasons to show that multinucleate fusions are primitive, but this is by no means always the case. The Zygomycetes, where multinucleate fusions are the rule, have progressed farther from their original algal ancestors than have the lower Oomycetes; and it is certain that the lower Ascomycetes, in which multinucleate fusions also occur, are a step in advance of both.

Of the relationship of the genus *Phytophthora*, Pethyb., to *Nozemia* and to *Pythium* nothing can be said until species of the last two genera be examined as to the development and cytology of their sexual organs. It is hoped to do this in the near future. It has been shown that *Phytophthora* and *Pythium* are very closely related, and it is equally certain on other grounds that *Nozemia* is very close to both. From considerations of its, on the whole, less specialized parasitism one would be inclined to expect it to go between the two. Looked at from the point of view of its sexual organs it is difficult to see how it can provide a link to connect *Phytophthora* to any form at present known. After much consideration of the matter the writer must acknowledge to being unable to find any similarity in the form of the sexual organs of *P. erythroseptica* to those of any other Fungus or Alga, near or remote; or any particular reason why they should be formed in that way; or any advantage in it. The Fungus itself gives no clue to its relationship. Particular attention was paid to the question whether or not fruit bodies of the normal type are ever formed, and the author is convinced from the examination of a more than usually large number of specimens that they never are. It is equally likely that no approach to the *Phytophthora* type will be found in the *Nozemia* group, although Hartig (19) in his description of *Nozemia Fagi* figures what was in all probability an amphigynous antheridium (see his Fig. 24*b*, l.c.) among many of the paragynous type. He, however, did not interpret it as such.<sup>1</sup> Working as he was with pieces of naturally infected beech seedlings, it is at least possible that this drawing was made from another Fungus which happened to be present. Provisionally it is necessary to regard *Phytophthora* as a strangely aberrant form which, having its origin somewhere near *Pythium*, has

<sup>1</sup> l. c., p. 49: 'Ausnahmsweise und zwar vielleicht dann, wenn in nächster Nähe des intercellularen Oogoniums kein anderweites Mycel sich findet, von dem aus die Entwicklung des Antheridiums erfolgen kann, schwilkt der Oogonenträger unmittelbar unter dem Oogonium blasig an und wird direct zum Antheridium, wie Fig. 24*b* zeigt. So deute ich wenigstens die zuweilen auftretenden Stellungen des Oogoniums auf dem Antheridium selbst.'

departed from the high road along which the remainder of the Peronosporales have progressed, and which leads to nothing else.

#### SUMMARY.

The oogonial incert grows through the already formed antheridium and forms an oogonium at the farther side, as Pethybridge described. The antheridium remains permanently clasping the oogonium stalk. The term 'amphigynous' is suggested to express this condition.

There is no fusion of male and female elements while the oogonial incert is within the antheridium.

The male and female organs are always borne on separate hyphae, but the Fungus is homothallic.

About two-thirds of the nuclei in both organs degenerate before division.

The nuclei in the oogonium and in the antheridium divide once only, mitotically and simultaneously. Those in the oogonium are arranged during this process in the form of a hollow sphere with one in the centre.

Zonation, the separation of the ooplasm from the periplasm, follows immediately after division. The ooplasm is uninucleate and is more hyaline than the periplasm, which is almost structureless.

The peripheral nuclei and one daughter of the central nucleus degenerate immediately after division, as do all those of the antheridium but one, probably.

A very large manocyst (receptive papilla) is found just as nuclear division is being completed protruding through the stalk of the oogonium into the antheridium.

When the manocyst is withdrawn a short fertilization tube grows in at the same place and delivers one male nucleus and the greater part of the cytoplasm of the antheridium to the oosphere.

The periplasm begins to disappear as soon as it is differentiated. It is all absorbed by the oosphere, the nuclei only being left outside. After fertilization there is no more left.

The spore wall consists of three layers, the primitive wall and the primary and secondary endospores. There is no exospore. The sexual nuclei do not fuse until the wall is mature.

The spore rests in the uninucleate condition.

The close relationship, cytologically, of *Phytophthora* to *Pythium* especially, and further to *Sclerospora* and *Plasmopara*, is pointed out. It is suggested that this series is a gradually ascending one, leading from the generalized type of sexual reproduction in the Acanthiales, where the whole of the contents of both organs unite to form the oospore, to the more specialized type of *Peronospora*, where a portion of the protoplasm of the oogonium remains permanently outside the oospore.

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## EXPLANATION OF PLATES II AND III.

Illustrating Mr. Murphy's paper on Sexual Organs of *Phytophthora erythroseptica*, Pethyb.

All figures were outlined by means of a camera lucida and the details were filled in afterwards. Figs. 1 to 35 inclusive represent a magnification of 1,200; Figs. 36 to 49, one of 1,800.

### PLATE II.

Fig. 1. General view of the sexual organs with a mature spore. Diagrammatic.

Fig. 2. Entrance of the oogonial inlet into the antheridium in longitudinal section.

Fig. 3. Further view of the same. The male and female organs are borne on separate hyphae.

Fig. 4. The same in transverse section. (a) A section through the lower part of the antheridium, showing the developing female organ inside. (b) A section through the upper part of the same antheridium, which the oogonial inlet has not yet reached. This is the same stage as is shown in Fig. 3 in longitudinal section.

Fig. 5. The oogonial inlet has reached the upper wall of the antheridium and is engaged in breaking through it.

Fig. 6. The piercing of the antheridial wall during the emergence of the oogonial inlet.

Fig. 7. The same in transverse section. The incept has grown obliquely through the antheridium and is emerging somewhat to one side. (a) The hypha which gave rise to the oogonial incept (b) The lower part of the incept with its antheridial sheath within the male organ. (c) The emergence of the oogonial incept.

Fig. 8. Further stage in the emergence of the oogonial incept.

Figs. 9 and 10. Stages in the development of the oogonium after emergence.

Fig. 11. A transverse section of organs of about the same age as in the last figure. (a) A section through the lower part of the stalk of the oogonium within the antheridium. (b) A section through the upper part of the stalk within the antheridium. (c) A section through the part of the oogonium which has emerged from the male organ.

Figs. 12 and 13. Further stages in the growth of the oogonium after emergence. Fig. 13 shows evidence of the rapid inward flow of the protoplasm.

Fig. 14. A transverse section through an antheridium of the same age as that shown in Fig. 13 with the stalk of the oogonium inside.

Fig. 15. The degeneration of nuclei in both organs before division. Tangential section.

Fig. 16. The same. The oogonium is now cut off by a thick plug in the stalk and the antheridium is cut off by septa.

Fig. 17. The arrangement of the nuclei during division.

Fig. 18. The same during the concluding phases. The nuclei have moved towards the periphery.

Fig. 19. The completion of nuclear division.

Fig. 20. The formation of the daughter nuclei. The zonation stage is about to set in.

Fig. 21. A longitudinal section through the sexual organs after nuclear division showing the manocyst or receptive papilla.

Fig. 22. The zonation stage in the oogonium. The functional female nucleus is seen in the centre.

Fig. 23. Another section showing the zonation stage and the degeneration of the peripheral nuclei.

Fig. 24. The formation of the fertilization tube.

Fig. 25. A longitudinal section through the sexual organs at the time of fertilization. (a) The fertilization tube containing the male nucleus. (b) The next section through the oogonium showing the female nucleus.

Fig. 26. A fertilization tube showing how the cytoplasm of the antheridium follows after the male nucleus.

Fig. 27. The stage just after fertilization, showing the male and female and the remains of the peripheral nuclei. The periplasm has disappeared.

### PLATE III.

Fig. 28. A longitudinal section through the sexual organs after fertilization. The oosphere has contracted somewhat, the remains of the fertilization tube are seen, and the antheridium is almost empty.

Fig. 29. A transverse section through an antheridium after fertilization, showing the almost empty condition and the presence of the stalk of the oogonium still inside.

Fig. 30. The formation of the oospore wall.

Fig. 31. Further stage of the same with the male nucleus in its characteristic position by the spore wall.

Fig. 32. A section through a spore with the wall practically full-grown. The nuclei are now in contact.

Figs. 33 and 34. Sections through mature spores showing the nuclei in the act of fusion.

Fig. 35. A section showing the outline of the oogonium and antheridium with a mature spore containing a fusion nucleus.

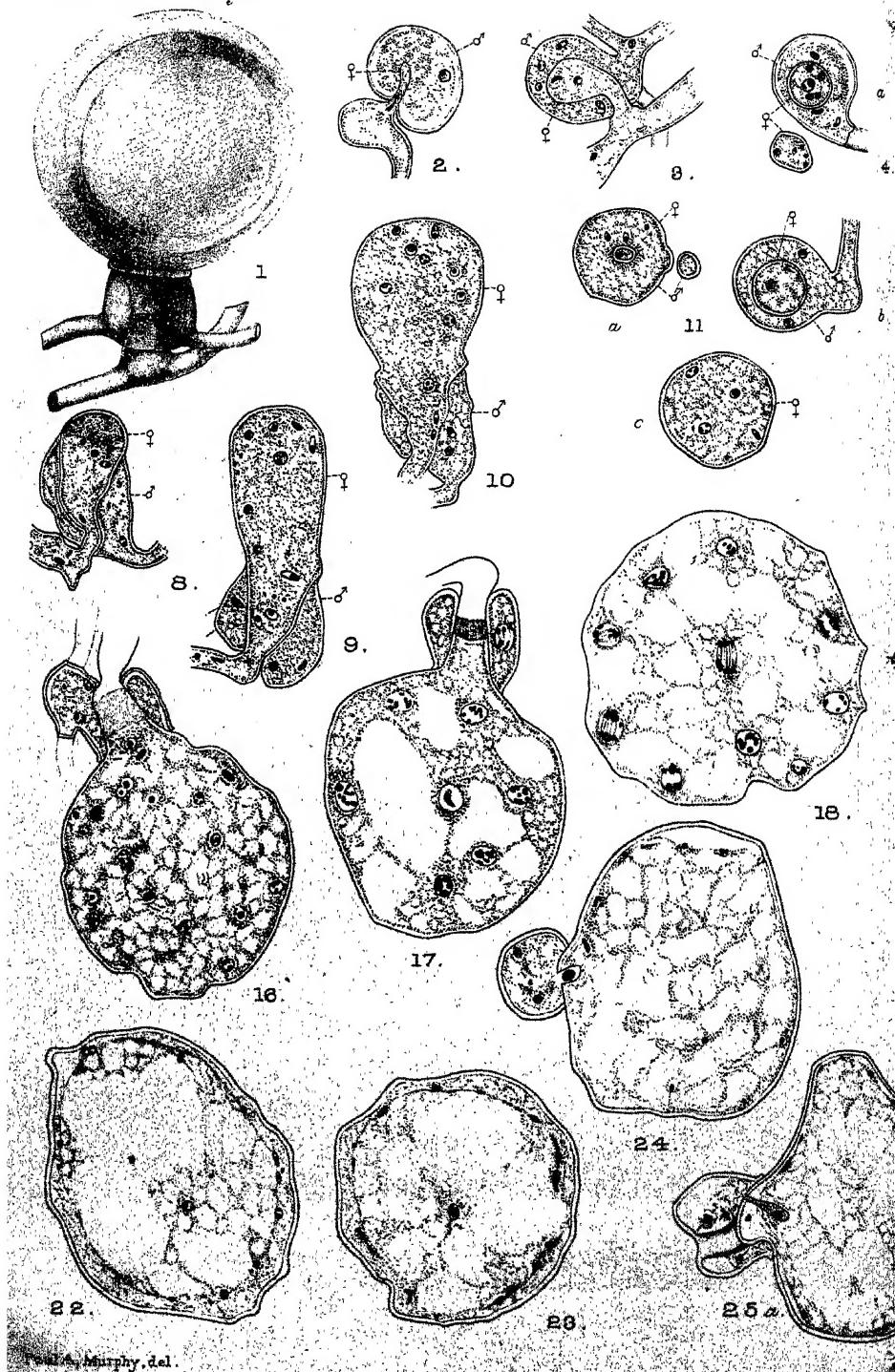
Fig. 36. One perfect nucleus (to right) and four nuclei showing various stages of degeneration, from the same oogonium.

Figs. 37 and 38. Spireme stages.

Figs. 39-41. The formation of the chromosomes.

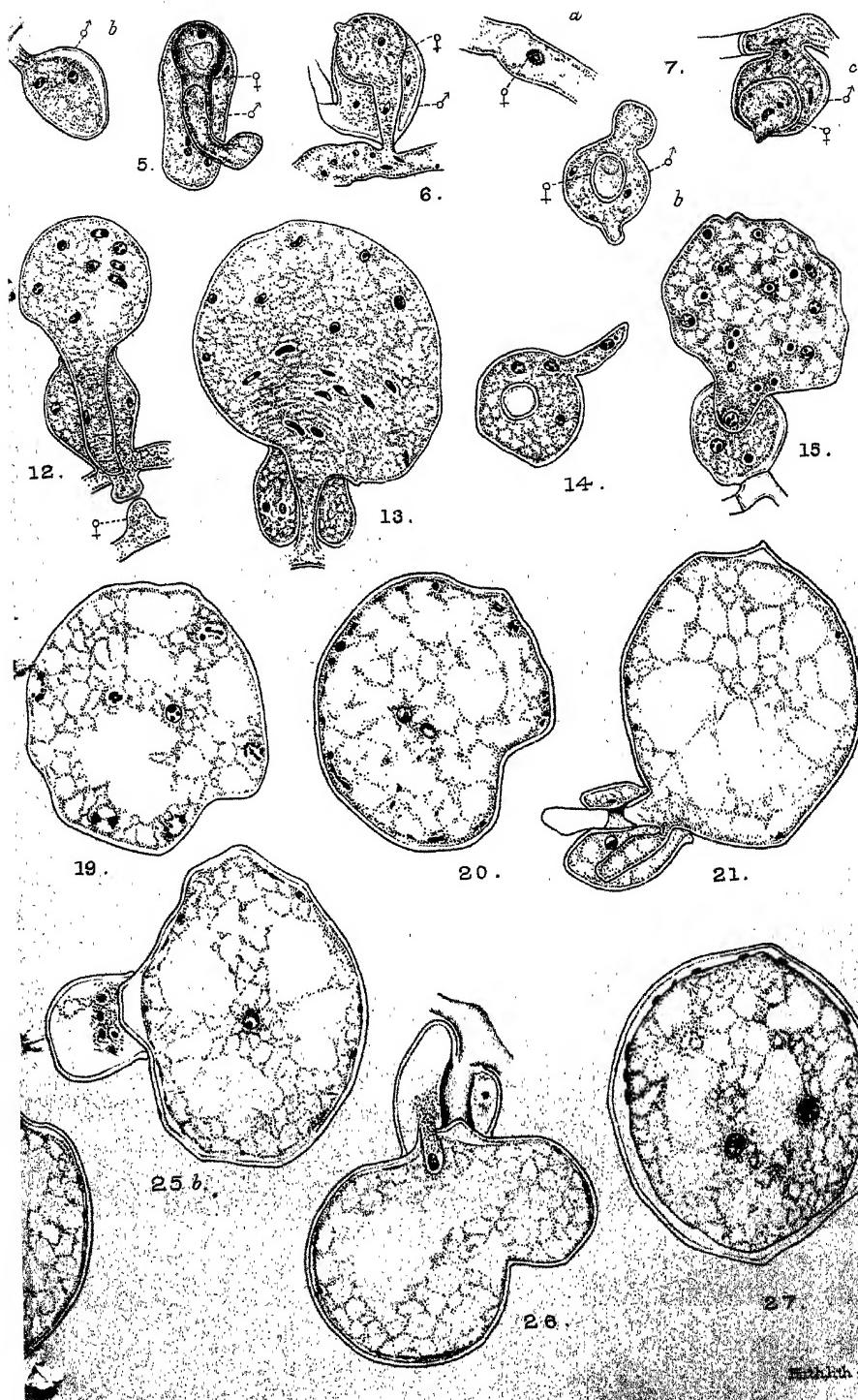
Figs. 42-48. Various stages of metaphase and anaphase.

Fig. 49. Telophase.

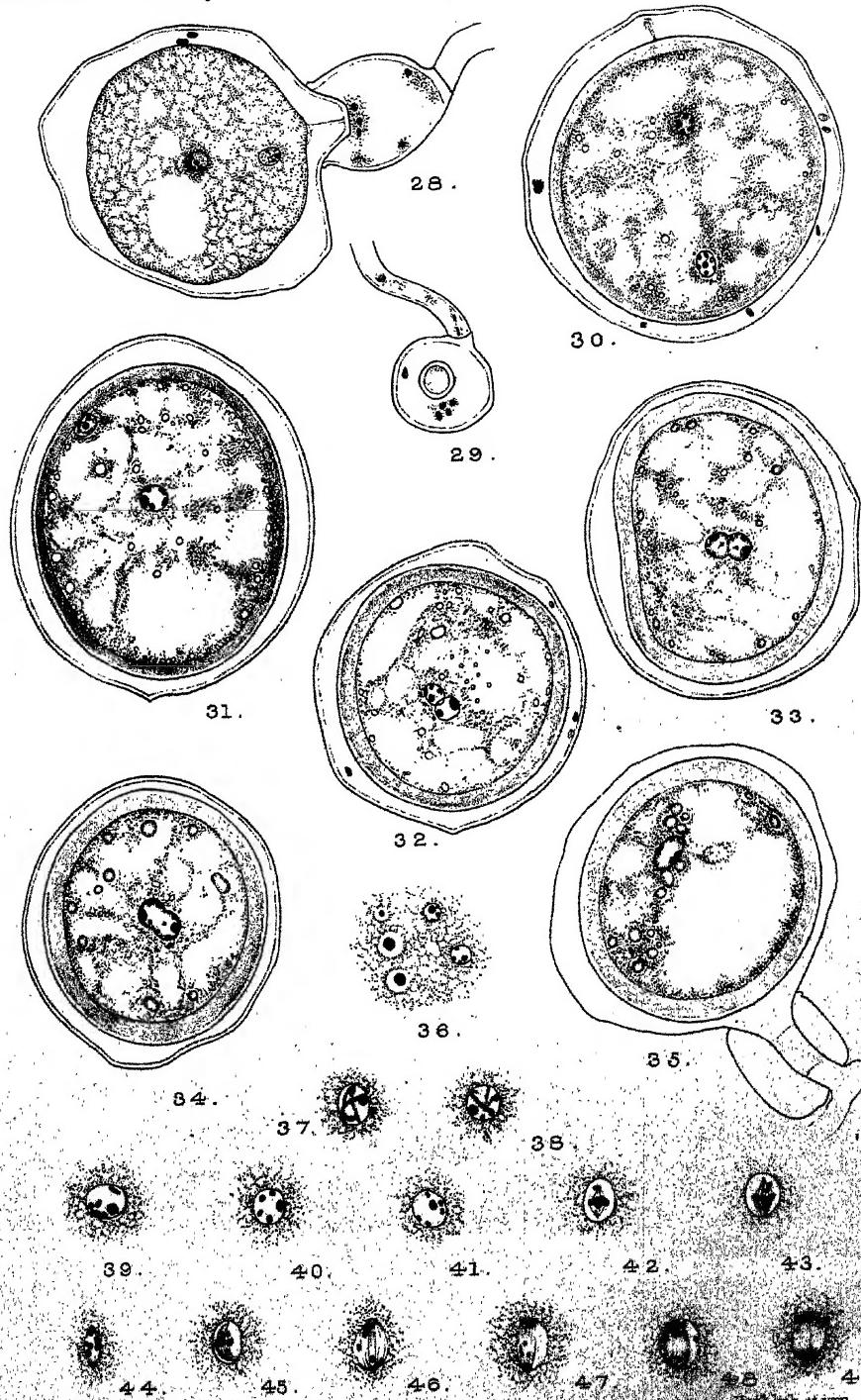


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MURPHY — PHYTOPHTHORA







Paul A. Murphy, del.

Betham etimp.



# The Development of *Thraustotheca*, a Peculiar Water-Mould.<sup>1</sup>

BY

WILLIAM H. WESTON, A.M., PH.D.

With Plates IV and V and two Figures in the Text.

THE genus *Thraustotheca* comprises at present the single species *T. clavata* (de B.), Humph., and is a rare and imperfectly known representative of the Saprolegniaceae. In the following paper the writer presents certain results of a culture study on the development of this fungus. These investigations were carried on in the Cryptogamic Laboratories of Harvard University under the direction of Dr. Roland Thaxter, for whose helpful criticism and advice the writer wishes to express his gratitude.

## HISTORY.

*Thraustotheca clavata* (de B.), Humph., was first discovered in 1880 by de Bary in a culture from algae collected at Vendenheim near Strassburg, and was named by him *Dictyuchus clavatus*, since its method of spore liberation resembled somewhat that of the genus *Dictyuchus* established by Leitgeb (19) in 1869. Under this name Büsgen (6) published the first description of the species in his study of sporangium formation in this and other Phycomycetes. In 1884 de Bary (3) mentioned the fungus as an example of endogenous cell formation, and in 1887 Rothert (24) briefly discussed the process in this and other forms. The first full description of the species, with illustrations, was given in 1888 in a posthumous paper of de Bary's (4) edited by Solms-Laubach. The latter suggested that certain peculiarities of the fungus, especially the disintegration of the sporangium membrane, might prove sufficient basis for establishing a new genus; and this was also urged by Fischer (11) in a later discussion of the species. Humphrey (15), although unable to secure *Dictyuchus clavatus* for study, considered it a species sufficiently aberrant to justify separation from the genus. On this species as a type he established the genus *Thraustotheca*, which has since been accepted by Schröter (26) and other systematic writers. A little more than thirty years after the fungus was first discovered

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by de Bary it was again brought to light by von Minden (21) in Germany, and by Coker and Hyman (9) for the first time in the United States. Von Minden made no detailed study of the form, but extended de Bary's description through his own observation of material from an inlet of the Elbe near Hamburg. By Coker and Hyman, however, the fungus, which had been collected from a pool at Chapel Hill, North Carolina, was studied for the first time in pure culture.

In September 1913 a luxuriant growth of *Thraustotheca* suddenly appeared in one of the writer's cultures which contained algae and silt taken from a spring-fed iron watering-trough near Great Barrington, Mass. Since the culture, although under observation for a month, had yielded only two common species of *Achlya*, the fungus was probably derived from an oospore.

#### METHODS.

The fungus was isolated and grown in cooled, covered battery jars. A little nutrient material was added every two weeks or so: flies or other insects and their larvae or eggs; bits of earthworms or salamanders; as well as the stems, leaf petioles, fruits, and seeds of various plants. By occasionally starting fresh cultures to offset the accumulation of injurious decomposition products, the fungus was thus maintained without difficulty for over eighteen months.

Pure cultures were obtained as follows: Hyphae bearing young sporangia were washed in several changes of sterile water, and allowed to remain in a drop on a slide until spores had been formed. This drop was then added to a few cubic centimetres of sterile water in an atomizer, shaken up, and the resulting suspension of spores sprayed on beef agar in large Petri dishes. After the plates had remained in a cool place for twenty-four to forty-eight hours, they were examined with a binocular microscope, and fragments of the young mycelia that proved to be uncontaminated by bacteria were cut out on small chips of agar, and transferred to fresh nutrient media.

Successful use was also made of the method devised by Klebs (18) and employed with unessential modifications by subsequent investigators, which involves the repeated transference of vegetative mycelium until bacterial contamination has been outgrown. In comparison with spraying the spores, however, this method proved to be unnecessarily tedious.

Cultures were also obtained from single spores under microscopic observation in the following manner: A few zoospores were introduced with a pipette into a drop of water on a slide, and a minute chip of beef agar was placed in the centre of the drop. Almost immediately the zoospores swam to the agar chip and came to rest here and there upon it. Under the microscope single zoospores on minute bits of agar were

isolated by the careful manipulation of a delicate platinum needle, and transferred to nutrient agar. This method proved more simple than the floating cover-glass method of Trow (29), Kasanowsky (16), and others, and quite as efficient. After pure cultures from single zoospores had been thus secured, stock cultures were maintained in flasks on fairly dry cornmeal mush, a medium in which the mycelia retained their vitality especially well.

An abundance of material in the several stages desired for study was secured by the methods of Klebs (18) and his successors, by growing mycelia in a variety of favourable liquid media and transferring them to sterile water or various solutions in which the development of the fungus was followed. Beef extract (1 to 3 per cent.), diluted beef juice, and filtered decoctions of split peas, yellow cornmeal, seed sweet corn, oatmeal, or other cereals were used. To suitable amounts of the medium in Petri dishes several bits of mycelium on agar were transferred; and after three to five days at laboratory temperature the resulting mats of mycelium were of sufficient size for use.

#### DEVELOPMENT OF THE FUNGUS.

In its development under normal conditions *Thraustotheca clavata* follows a regular cycle, since the mycelium gives rise first to sporangia and later to sexual organs. For convenience, therefore, the successive stages will be considered in the order of their appearance.

*Mycelium.* In both pure and gross cultures the mycelium consists of a number of main hyphae which grow out in a radial direction, and by branching and rebranching give rise to a thick mat of interwoven filaments. Certain variations in size, shape, structure, and method of branching characterize the hyphae in different nutrient solutions; but since these variations have already been described by Klebs (18), Kauffman (17), Horn (14), and Obel (22), for several Saprolegniaceous species, they need not be discussed here.

In pure cultures the hyphae frequently contain masses of pectin which vary in extent from knobs or bosses on the inner wall surface, and from discoid or cylindric septa (Pl. IV, Fig. 37) to lobed structures occupying a large part of the lumen. Frequently also portions of the hyphal content may separate, become surrounded by a delicate wall, and then resume growth to form endogenous hyphae, which often grow in opposition to each other until one or both may be forced out through the original hyphal wall. Under favourable conditions both the erumpent hyphae and those still *in situ* may form normal sporangia (Fig. 35).

The formation of both pectin masses and endogenous hyphae seems in the case of *Thraustotheca* to result from localized mechanical injuries as well as from the effect of ionized metals or plasmolysis, which Horn (14) found to cause similar phenomena in *Achlya polyandra*, de B.

*Sporangia.* Typical sporangia of *Thraustotheca* are broadly clavate in shape, and lack papillae of dehiscence. Variations from the type to a fusiform or subspherical shape may occur; and in size the sporangia may show all gradations, from the largest, about 300 by 60  $\mu$ , containing several hundred, to small impoverished specimens containing only a few spores. Renewal of the sporangia takes place not only by cymose branching, the only method heretofore noted, but also by successive formation in basipetal series (Fig. 27).

Formation of sporangia by the mycelium follows a diminution of the food supply in accordance with the general law established by Klebs (18). Since certain important details in the process of sporangium formation could not be made clear by serial sections, a study of the living material was made. The following description is derived from observations on a large number of sporangia in hanging-drop cultures under a Zeiss J. water-immersion lens.

The process begins with a gradual accumulation of protoplasm and a proportionate enlargement of the hypha, generally at the tip (Fig. 1). Eventually the accumulating content fills the sporangium initial, leaving at most only a small axial vacuole (Fig. 2). A septum is now formed across the sporangium base. The granular protoplasm in this region suddenly separates, leaving a hyaline zone (Fig. 3). At the bottom of this zone the septum is formed (Fig. 4), while at the periphery the lateral sporangium wall is reinforced, and in a few moments the granular content moves back, obliterating the hyaline area. Because of its reinforcement (cf. Fig. 29) the basal part of the sporangium wall is especially persistent, a peculiarity of the genus noted but not explained by previous investigators. Occasionally the septum shows columella-like outgrowths into the sporangium (Fig. 36); but as a rule the statement of Solms-Laubach and others that the septum is strongly bulged out into the sporangium is not confirmed.

The content now undergoes differentiation into a number of spores. The first step in this process is the separation of the protoplasm into spore initials. Irregular clefts filled with cell-sap (Fig. 6) appear in the centre of the sporangium, and extend (Fig. 7) to form a system of intersecting lacunae (Fig. 8) which divide up the sporangium contents into roughly polygonal spore initials. Since the sporangium content, in spite of this division, contracts as a whole when plasmolysed, we may infer that a thin peripheral layer of protoplasm still remains uncleft.

A rapid change in the appearance of the sporangium now indicates that a second phase in its development has begun. The content suddenly becomes translucent and temporarily vacuolate, while the individual spore initials become less distinct than heretofore (Fig. 9). This is probably the result of a loss of cell-sap with a consequent shrinkage of the sporangium

and contraction or compression of the spores. At any rate, comparative measurements demonstrate that the sporangium has distinctly decreased in size. Since Rothert (24) in *Saprolegnia* saw the clefts extend and rupture the remaining peripheral layer of protoplasm, we may infer that in the present instance also this rupture has taken place, allowing the escape of cell-sap from the clefts with a consequent shrinkage of the sporangium and partial obliteration of the lacunae of demarcation between the spores. The translucent and vacuolate condition of the content is of brief duration, and there seems no need of regarding the vacuolation as marking a separate stage in sporangium development.

In the final phase, which next ensues, the spores, now clearly granular and surrounded individually by delicate membranes, imbibe water and swell, becoming more and more separate (Fig. 10). It is to be noted that the sporangiospores have maintained their identity from the beginning of the process. As Figs. 6-13 show, the completed spores occupy the identical positions of the spore initials when first marked off by the clefts. There is, moreover, no evidence to support the assertion of Büsgen (6) and others that the spore initials fuse to a homogeneous mass.

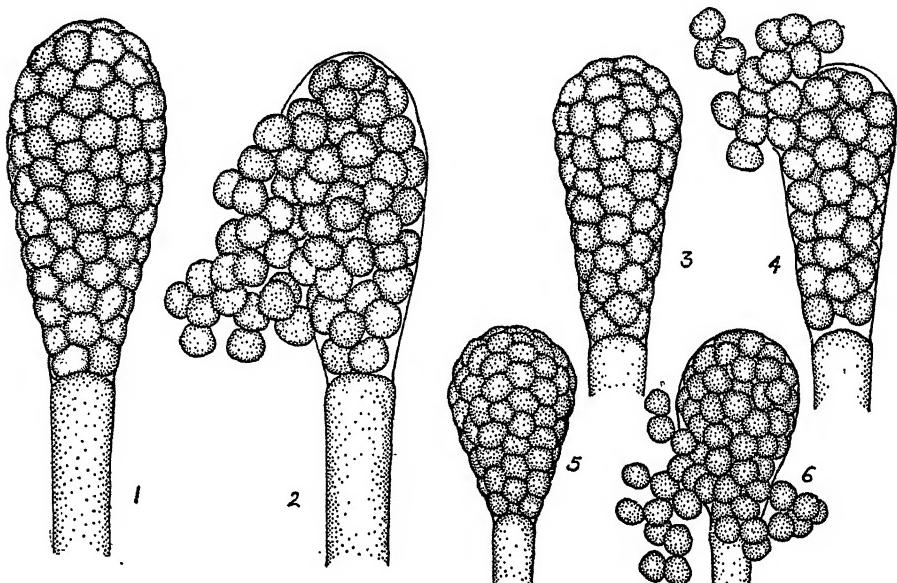
The foregoing description applies particularly to the densely filled sporangia; but in the less common axially vacuolate sporangia, as well as in certain abnormal sporangia (Fig. 38) which are characterized by large central vacuoles and thin peripheral protoplasm, and which develop at a temperature near the maximum ( $31-32^{\circ}$  C.), spore-formation is also associated with the centrifugal extension of cleavage furrows. If, however, the sporangium wall is punctured just as cleavage begins, both the protoplasm that escapes and that which remains undergo division into irregular spore-masses by an incomplete cleavage from the surface inwards (Figs. 40 and 41).

It is to be noted that the process of sporangiospore formation in *Thraustotheca* presents no evidence of an intersporal substance, but agrees in the main with the descriptions of Rothert (24) and Humphrey (15) for other Saprolegniaceae. The assertion of de Bary (4) and of von Minden (21) that the sporangiospores of *Thraustotheca* are embedded in a 'Zwischen-substanz' is probably based on Büsgen's misinterpretation of the process of spore formation in the species, rather than on individual observation of living materials.

*Liberation of the Sporangiospores.* The method of spore liberation in *Thraustotheca* differs from that which has been described for any other genus of the Saprolegniaceae. The papillae of dehiscence which characterize most members of the family are lacking, and the non-motile sporangiospores escape by bursting the sporangial wall. At high temperatures ( $30-31^{\circ}$  C.) occasional sporangia develop apical papillae (Fig. 38) which closely resemble the normal escape papillae of other genera. In

these abnormal cases, however, escape of the sporangiospores also takes place by rupture of the wall near the papilla or elsewhere (Fig. 39).

When the individuality of the spores is finally established, they are distinctly polygonal; and the sporangium wall presents a plane, even contour (Fig. 10). Gradually, however, the spores imbibe water, swell, and become spherical; and as their flattened surfaces of mutual contact round out, the spores are separated, and the sporangium wall becomes tightly stretched over their bulging outer surfaces. As this process of swelling and rounding goes on, the restraining wall becomes increasingly



TEXT-FIG. 1. Escape of the sporangiospores. From camera lucida drawings of living material. 1, 2. A sporangium which burst at the side, allowing a large number of spores to escape. Note adherence of the spores. 3, 4. A small sporangium just before and just after bursting at the tip. 5, 6. A short, broad sporangium from which a number of spores were forced out, rupturing the wall near the base.

distended and eventually bursts (Fig. 13). Liberation of the spores does not take place at once. When the membrane is ruptured, the spores in the immediate region of the break are forced out, relieving the pressure. The elastic membrane, however, still restrains the remaining spores, and after a few minutes the distension among these may become sufficient to push out those most favourably situated.

The point at which the bursting of the sporangium membrane takes place varies according to conditions (Text-fig. 1). When the sporangia are long and slender, the longitudinal expansion, augmented by the upward bulging of the sporangial septum, far exceeds the lateral expansion, and the sporangium membrane is generally ruptured at the tip. In broadly clavate

or spherical sporangia, although it is sometimes possible from the arrangement of the spores to predict where they will be set free, the dehiscence is quite irregular. Moreover, the wall often varies in thickness, and the weakest spots are naturally the first to give way. Occasionally, where the thickened basal collar merges into the normal sporangium wall, there appears to be a line of weakness along which rupture takes place. In the case of intercalary sporangia, the thick septa at the ends prevent any longitudinal expansion, and discharge is invariably lateral (Fig. 27).

During sporangium dehiscence, a distinct adhesion and mutual attraction is noticeable among the spores. Frequently sporangiospores remain sticking to hyphae with which they have come in contact when discharged. Microchemical tests fail to demonstrate any intersporal slime such as Swingle (28) found excreted from the spores of *Rhizopus*; and since this adhesive quality is soon lost, it is probably due only to the viscosity of the recently formed cellulose walls. At the moment of discharge, however, the spores seem to exert a mutual attraction; since they do not immediately scatter, but press together, sliding over each other and changing their relative positions, but still keeping in contact. It is of interest to note that the contiguous surfaces of such spores are often decidedly flattened, as if through the exertion of mutual pressure.

The fragility of the sporangium wall has been over-emphasized by previous investigators, probably because they did not observe the actual bursting of the sporangium, and hence inferred that the spores were freed by disintegration of the wall. In pure culture the sporangium walls are as persistent as would be the walls of any other Saprolegniaceous sporangium that had undergone a like distension and rupture.

It seems advisable at this point to call attention to the confusion which often arises from an unrestricted use of the terms 'zoospore' or 'spore' in the case of diplanetic Saprolegniaceae. To avoid any misunderstanding in the case of *Thraustotheca* the term 'sporangiospore' will be used for the non-motile spores formed in the sporangium: the motile spores emerging from these will be called 'zoospores', and the encysted spores into which these in turn metamorphose will be called 'cystospores'.

The liberated sporangiospores (Fig. 13) vary from a rounded polygonal to a practically spherical shape; their average diameter is about  $12.5\ \mu$ ; and the distinct wall is about  $0.5\ \mu$  in thickness. The content consists of a number of microsomes scattered irregularly through a finely granular, almost hyaline matrix, a less densely granular central region indicating the position of the nucleus.

*Further History of the Sporangiospores.* The sporangiospores may either emit motile zoospores, give rise to hyphae of germination, or form dwarf sporangia, according to certain conditions of the environment. In

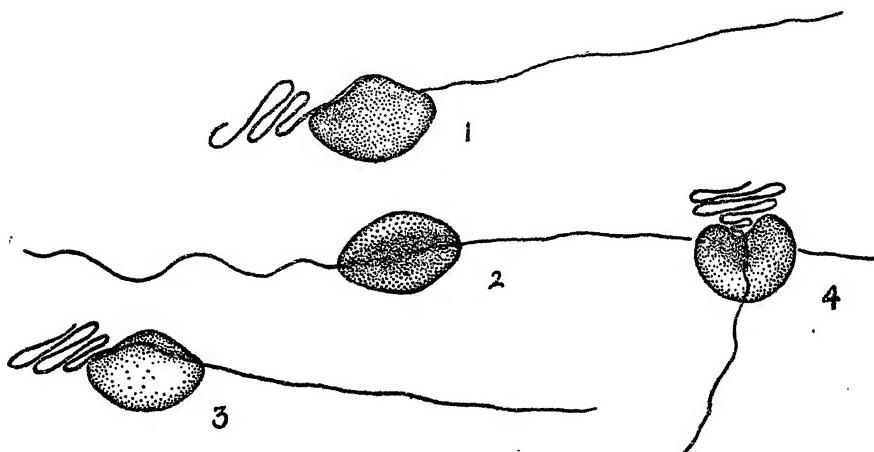
pure water, zoospore emergence takes place ; in the presence of nutriment, germ tubes are formed ; and when, after hypha-formation has begun, the available nutriment is suddenly exhausted or removed, dwarf sporangia are produced. Frequently, also, substances which increase the osmotic pressure, but are without nutrient value, seem to exert the same influence as nutriment in this connexion.

Zoospore emission occurs as follows : The sporangiospore content emerges rapidly through a softened papilla of escape as an amorphous mass (Fig. 15), which assumes the characteristic structure of the motile zoospore. Near the already apparent nucleus (Fig. 16) a contractile vacuole develops, while the surface becomes grooved by a depression (Fig. 17) from which, close to the nucleus, arise two delicate cilia that lash slowly back and forth. This lashing increases in violence until the zoospore finally frees itself with a sudden jerk, and swims actively away (Fig. 18). In the case of sporangiospores retained *in situ*, the emerging zoospores are imprisoned by the enveloping sporangium membrane or by surrounding sporangiospores. As a result, sporangiospores, zoospores, and cystospores may at times be observed within the same sporangium wall (Fig. 29). From retained sporangiospores in contact with the sporangium wall, zoospores often emerge through the wall as in the genus *Dictyuchus* (Fig. 30).

The zoospores are of the laterally biciliate type found in most Saprolegniaceae. Under high magnification the inadequacy of the commonly used terms 'bean'- or 'kidney'- shaped becomes obvious. Precise description is difficult, but the zoospores may be characterized roughly as subovoid and as bearing a longitudinal furrow. Since the customary figures of this type of zoospore are somewhat misleading, an attempt has been made to present the solid form in the accompanying diagrams (Text-fig. 2). In size the zoospores average about  $12.5\mu$  in length and  $9\mu$  in width. Their locomotion, involving progression and rotation on the long axis, is in an irregular spiral course through the water. The more tapering end is directed forward, and by the tractile action of the shorter anterior cilium the zoospore is drawn rapidly along, while the longer cilium is dragged behind as a more or less passive rudder. Although rotation is generally to the right, it may be reversed or may cease, while the zoospore moves irregularly forward. These changes in motion seem to be dependent upon the activity of the posterior cilium, although the precise method of lashing is not distinguishable. To chemical and other stimuli the zoospores respond much as did the *Saprolegnia* zoospores investigated by Pfeffer (23) and Stange (27). Duration of the motile period is not fixed. A tendency to motility, together with a sensitiveness to those influences of the environment that result in encystment, is inherent in each zoospore. In pure water a zoospore may swim about for 30 or 45 minutes, but at any minute, if

nutrient solutions, or even non-nutrient solutions which increase the osmotic pressure, are allowed to diffuse into the water, encystment immediately occurs. The zoospore becomes quiescent, and contracts to a sphere, while the contractile vacuole disappears and large granules appear in the finely granular content. Usually the cilia, still bending actively into twisted loops (Fig. 19), are dropped off during contraction of the zoospore ; but occasionally they seem to deliquesce into minute droplets and disappear.

The cystospore thus formed is, in contrast to the sporangiospore, almost exactly spherical in shape, about  $10\ \mu$  in diameter, and possesses a more sharply defined wall, enclosing a denser content. Germination, which



TEXT-FIG. 2. Zoospores. Diagrams showing the characteristic shape. From camera lucida drawings of living spores. 1. Side view, the active anterior cilium at the left, the trailing posterior cilium at the right. The groove (above) cannot be seen. 2. From above, looking down on the groove, anterior cilium at the left. 3. Oblique, posterior-lateral view. The groove is partly shown. 4. Posterior view showing groove and position of cilia.

may occur at once or after some weeks of inactivity, is invariably by the formation of a hypha (Fig. 20). In hanging-drop cultures the growth of this hypha into a bit of fly muscle, the formation of rhizoids, and the ultimate development of a new plant can easily be observed. Since very little food material is present, the resulting plants are small (about  $300-400\ \mu$  in length), and bear sporangia containing only a few spores (Fig. 22). Occasionally, also, the hypha of germination may form a dwarf sporangium (Fig. 23) similar to those which arise from the sporangiospores, and will be described later.

The sporangiospores form hyphae of germination in either solid or liquid media. Rapid germination and vigorous growth to a mycelium occur when sporangiospores are transferred to beef decoctions, cornmeal mush, and cylinders of potato or carrot. Under these conditions the spore becomes vacuolate, swells rapidly (Fig. 24), and sends out a tube (Fig. 25)

which eventually forms a mycelium. In the case of sporangiospores retained in unemptied or partially emptied sporangia, the germ-tubes make their way through the enveloping sporangial wall (Fig. 31), a condition presenting close resemblance to the doubtful genus *Aplanes*.

The development of dwarf sporangia from the sporangiospores is shown in Figs. 26, 28, 32, 33, and 34. Within these dwarf sporangia are formed two or three spores somewhat smaller than the normal. Since the total expansion of so few spores is insufficient to rupture the sporangium wall, the spores remain *in situ*, and emit their zoospores through the enveloping membrane (Figs. 26 and 34). These zoospores differ from those already described only in their smaller size and shorter motile period. This phenomenon of dwarf sporangium formation presents an interesting analogy to the production of secondary conidia in the case of certain Entomophthoreae and Peronosporaceae.

*Gemmae.* In *Thraustotheca* gemmae are formed when environmental conditions inhibit the development of reproductive organs but permit vegetative growth. By maintaining vigorous mycelia at a temperature (31–32° C.) near the maximum, or in harmless salt solutions at concentrations suppressing the formation of other organs, the formation of gemmae is easily induced. Under these conditions porangium and oogonium initials, as well as portions of the hyphae, are walled off and become inactive. The gemmae thus formed may be clavate (Pl. V, Fig. 42), spherical (Figs. 43, 46, and 48), cylindrical, or irregular (Fig. 47) in form, and show variations in density of content and thickness of wall. They cannot survive desiccation; and since their metabolic activity, though diminished, still continues, their endurance is proportionate to the amount of protoplasm they contain.

When inhibitory conditions are removed, the gemmae resume their growth and give rise to vegetative hyphae (Fig. 48), sporangia (Figs. 42 and 43), secondary gemmae (Figs. 45 and 46), or sexual organs. In this connexion the initial tendency of the structure from which the gemmae arose has no influence; since any gemma may produce any type of vegetative or reproductive structure—the process being governed by the same rules which apply in the development of these structures from the mycelium.

In view of the conditions under which the gemmae arise, and of the lack of physiological and morphological fixity which characterizes them, Maurizio's (20) opinion that they should be regarded as phylogenetically significant seems hardly justifiable. On the contrary, in the present instance, as in the case of *Saprolegnia mixta* (cf. Klebs, 18), they appear to be 'Hemmungsbildungen' representing only a transient resting-state induced by unfavourable conditions.

The sexual reproduction of *Thraustotheca* closely resembles that of the *prolifera* group of the genus *Achlya*.

*Antheridia.* The antheridial filaments arise at intervals along the

main hyphae as slender secondary branches (Fig. 53). They are usually gently curving (Figs. 49, 52, and 53), and do not present the irregular crooked appearance which Coker and Hyman (9) regarded as characteristic. As a rule, when the antheridial branches grow through the mycelium, they either come in contact with the developing oogonia or lie in close proximity to them. When this occurs the branches form antheridia (Figs. 49 to 54)—irregular, cylindrical organs, either simple or branched, which curve around the oogonium in close contact with its surface. If they fail to reach the oogonia, however, the filaments often give rise to zoosporangia (Fig. 52). Moreover, antheridia are never formed free, even in cultures containing phosphates or other salts, which Klebs (18) found to stimulate the formation of antheridia on the oogonia. These observations seem to indicate that antheridium formation is a response to some contact or perhaps chemical stimulus furnished by the young oogonia. This stimulus causes no response unless the filaments are very near the oogonia; but usually the extensive branching of the filaments ensures this result. Since the one to several antheridia attached to each oogonium are either of androgynous (Fig. 53) or diclinous (Figs. 49 to 52) origin in cultures from a single spore, the fungus is evidently monoecious.

*Oogonia.* The oogonia usually arise somewhat later than the antheridial filaments. Although generally borne in a racemose manner on the tips of short lateral branches (Figs. 49 and 53), they may also occupy a terminal or intercalary (Fig. 51) position on the main hypha. In any case, they are formed by the accumulation of contents, swelling of the hypha, and formation of a septum or septa, much as in the case of the sporangium initials. The shape, however, is regularly spherical, although the intercalary oogonia are occasionally barrel-shaped. At an early stage in the development of the oogonia, at times even before the septa have been formed, the antheridial filaments become attached and form antheridia upon them. When this has taken place the coarsely granular content of the latter is modified to innumerable globules embedded in a finely granular matrix. The formation of oosphere initials now takes place (Fig. 54) by a progressive separation of the contents from the centre outward, to form bluntly pyramidal masses which round off and become surrounded by walls (Fig. 55).

The formation of oospheres in an oogonium seems in some way correlated with the attachment of antheridia thereto. If no antheridia are formed on a young oogonium, it does not form oospheres, but gives rise, often repeatedly, to stalked or sessile secondary oogonia by proliferation (Fig. 45), or forms sporangia (Fig. 49). On the contrary, when once an oogonium has acquired antheridia, it does not give rise to sporangia or secondary oogonia, but forms oospheres. Moreover, separation of the oogonial content into oospheres in no case takes place until after attachment

of the antheridia. No exception to the foregoing condition has been noted in a large number of cases ; and the figures of Coker and Hyman (9, Fig. 8) and of Horn (14, Fig. 19) in the case of *Achyla polyandra*, de Bary, strikingly corroborate the writer's observations on this point. The parthenogenetic formation of oospheres would, of course, be a serious objection to this interpretation. No such cases were observed, however, under the usual culture conditions, nor could parthenogenesis be induced by the method Trow (29) found successful in the case of *Achyla americana* var. *cambrica*.

When the oospheres have been formed, the oogonium wall, especially when turned red by chlor-iodide of zinc, is seen to be smooth, thick, and definitely pitted. The character of the pitting varies under different conditions, since, on impoverished mycelia, the oogonia show the weak pitting (Figs. 49, 53, 63, and 64) which has been considered of diagnostic value by previous investigators, while on vigorous mycelia the oogonial walls are marked by large and numerous pits (Fig. 51). It is of interest to note that Horn (14) found a similar variation in the pitting of oogonia in *Achyla polyandra*, de Bary.

After the oospheres are differentiated, they undergo a period of maturation (Figs. 54 to 58), the wall increasing in thickness and the protoplasmic contents separating out as the oil droplets fuse together. Finally the structure of the mature oosphere is attained, characterized by a thick, unroughened wall within which the protoplasm is condensed into a bowl-shaped granular mass partly surrounding a large, slightly flattened oil globule (Fig. 58). This eccentric arrangement of the oospore content may persist for months ; hence, in the case of *Thraustotheca* at least, Maurizio's (20) objection to the term eccentric as representing only a transient condition seems groundless.

Among the maturing oospheres, especially after staining, can be seen slender fertilizing tubes which arise from the antheridia, and enter the oogonia through the pits in the wall (Figs. 50 and 51). In contrast to the thickened antheridial walls, which show the same composition as those of the oogonia, the delicate fertilizing tubes are of cellulose. It is worthy of note that the fertilizing tubes were never seen to penetrate the oospheres, nor was any sudden passage of material down the tubes observed.

*Germination of the Oospores.* Germination of the oospores of *Thraustotheca* has not been observed by previous investigators. Germination was induced by the writer, however, by placing mature spores in pure water maintained at a temperature optimum for growth (*circa* 25° C.). Under these conditions the protoplasmic content increases in extent, probably at the expense of the oil globule, which becomes irregular (Fig. 59) and gradually disappears. The wall, meanwhile, first swells (Fig. 59), and is resorbed and distended until it becomes comparatively thin (Fig. 60). As a result of these modifications the resting oospore, of about 17  $\mu$  in diameter,

becomes transformed into a spherical body 22 to 24  $\mu$  in diameter, containing a loose protoplasmic reticulum (Fig. 60). A hypha (Fig. 61) is now sent out, which may, after limited growth, form a sporangium, or may continue to grow into an extensive mycelium depending on the amount of nutriment stored in the spore or present in the surrounding medium. In pure water sporangia are formed, either directly or on short hyphae (Fig. 63); but if nutriment is added, a more or less extensive mycelium arises (Fig. 62). Since the heavy wall of the oogonium persists in pure cultures, the oospores germinate *in situ*; and although in some cases their expansion is sufficient to rupture the enveloping wall, in general the hyphae of germination grow out through the pits, or follow along the empty shells of the persistent antheridia until free (Figs. 62, 63, and 64).

#### DISCUSSION.

The development of *Thraustotheca* presents several points of interest.

*Spore liberation.* The method of sporangium dehiscence and spore liberation which characterizes the genus is at present unique among the Saprolegniaceae. In no other member of the family do non-motile sporangiospores escape by bursting the sporangium wall. Moreover, none of the theories advanced in explanation of spore liberation in other genera seem applicable to the process in *Thraustotheca*. The theory that the sporangiospores escape by their own motility is not corroborated, since they are consistently non-motile and without cilia. Even in the better-known genera, however, this theory is not universally supported. In *Saprolegnia*, *Leptolegnia*, and *Pythiopsis* the sporangiospores are undeniably ciliate and motile. Humphrey (15), however, believed that an internal force other than spore motility was involved in emptying the sporangia; and the writer has observed a number of cases which corroborate this point of view. In *Pythiopsis cymosa*, de Bary, for instance, dissolution of the escape-papilla is occasionally delayed until the sporangium becomes so distended by the swelling of the spores that it finally bursts at the tip, expelling the zoospores a considerable distance with such violence that they seem stunned and remain quiet some minutes before swimming away. Moreover, although the sporangiospores of *Aphanomyces* escape from the sporangium and group in a hollow sphere at its mouth, both de Bary (2 and 4) and Rothert (24) found that they lack demonstrable cilia. In the case of *Achlya* certain investigators have asserted that the escaping sporangiospores are ciliate, while others have maintained that they are not. Cornu (10) first claimed that the sporangiospores are ciliate; and this has been corroborated by Hartog (12) for *Achlya polyandra*, Hild., and *A. recurva*, Cornu; by Humphrey (15) for *A. americana*, Humph.; and by Coker (8) for *A. paradox*, Coker, and *A. caroliniana*, Coker. On the other hand, Rothert (24), Horn (14), and Coker (7) deny the existence of cilia on the sporangiospores.

of *Achlya polyandra*, de Bary. This disagreement may possibly indicate that ciliate sporangiospores are developed only in some species of the genus, or that they are developed in the same species only under certain conditions. In any case, in view of the conditions in *Thraustotheca*, *Aphanomyces*, and *Achlya*, the assumption that automotility of the sporangiospores in the Saprolegniaceae is the prime factor in their escape may well be questioned.

To a second theory, also, that the sporangiospores are forced out of the sporangium by the swelling of some gelatinous substance, no support is given by *Thraustotheca*. No such substance is demonstrated when spore liberation takes place in a suspension of India ink particles or in the presence of various stains. Moreover, in the other genera of the family this theory is not consistently supported. To be sure, de Bary (1), who first promulgated the theory for *Achlya prolifera*, remained unshaken in his conviction throughout his long investigations, and Coker (7) corroborates him in the case of *Achlya de Baryana*, Humph., while Rothert (25) maintains that such an expulsive substance is present also in *Aphanomyces laevis*, de Bary. It must be noted, however, that de Bary and Rothert did not demonstrate this substance, but inferred its existence from the behaviour of the escaping spores; while Coker further maintained that the persistence of the spores in groups showed them to be still embedded in the substance which caused their expulsion, an interpretation which the normal adhesion of the spores by their viscid surfaces leads one to accept with reserve. Furthermore, Hartog (12) and Humphrey (15) were unable by seemingly conclusive tests to demonstrate such a substance in the case of *Achlya*; while Rothert (24) offered somewhat less convincing evidence for *Saprolegnia*. The expulsive material was generally supposed by Büsgen (6) and other investigators to be an intersporal substance derived from the gelatinization of the 'cell plates' which they thought were formed between the spore initials. Walz (30), however, led by his observations on certain algae, maintained that gelatinization of the inner layers of the sporangium wall formed the expansive material which expelled the spores. In the case of *Thraustotheca* no evidence is found to support either of these views.

A third theory was advanced in 1851 by A. Braun (5), who maintained that the contraction of the elastic sporangium wall, which had been distended by the swelling of the zoospores within, was instrumental in emptying the sporangium. With certain modifications this early theory seems applicable to the process of sporangium dehiscence and spore escape in *Thraustotheca*. As has been said, the sporangiospores lack cilia, and no expulsive substance is apparent; but there is no doubt that the sporangiospores swell, and distend the enveloping wall. It is obvious that even the slight increase in the size of the individual spores would, in large masses, produce a considerable aggregate increase in the sporangium volume and a proportionate distension of the wall. On the whole, the swelling of the sporangiospores against the

elastic restraining membrane, combined with the mutual attraction and superficial viscosity of the newly-formed spores, seems to explain quite adequately the sporangium dehiscence and spore liberation that characterizes *Thraustotheca*. Moreover, it is probable that the factors which affect spore liberation in this genus may play at least a contributory part in other cases.

*Mutual Attraction of the Sporangiospores.* The escaping sporangiospores of *Thraustotheca* show a mutual attraction resembling that which in a more pronounced form causes the aggregation of *Achlya* sporangiospores into a hollow sphere. Hartog (12 and 13) was the first to maintain that this phenomenon in *Achlya* was due, not to the embedding of the spores in a globule of gelatinous material, but to 'adelphotaxy', a form of irritability which he defined as 'the tendency of spontaneously motile cells to assume definite positions with regard to their fellows'. As defined, this term is not applicable to the non-motile sporangiospores of *Thraustotheca*. Since, however, the ciliation and auto-motility of *Achlya* is not indisputably established, and since in *A. paradoxa*, Coker, the escaping sporangiospores show only an irregular aggregation quite comparable to *Thraustotheca*, it seems justifiable to extend the term to include the phenomenon in this genus.

The precise nature of adelphotaxy is at present unknown. It depends seemingly on the life of the spores; since Humphrey (15) found that the spores of *Achlya* fell apart when killed with osmic acid fumes at the precise moment of emergence, and the writer has found a like condition to obtain in *Thraustotheca*. This mutual attraction, moreover, is effective only when the spores are in contact or separated by a very minute space. In *Thraustotheca*, sporangiospores separated during escape by more than one half their diameter remain apart; and in *Achlya* Hartog (12) and Coker (8) have noted that an escaping sporangiospore, if separated from its fellows by its length, fails to take position in the hollow sphere, but moves off. Sharp distinction must be made, however, between adherence of the sporangiospores from mutual attraction and from the glutinous character of the newly-formed walls. In both *Achlya* and *Thraustotheca* the attraction is of brief duration; but the adherence of the hardening walls of the appressed spores holds them firmly even after they have emitted zoospores, and are but empty cysts.

*Relationship.* Although the exact relationship of *Thraustotheca* is largely a matter of conjecture, certain facts seem of significance in this connexion. A comparison with *Dictyuchus* gives scant basis for the customary taxonomic association of the two genera. The sporangia, both in structure and in method of spore liberation, are distinctly different; while the sexual organs of the three authentic species of *Dictyuchus* are quite unlike those of *Thraustotheca*. To *Achlya*, however, *Thraustotheca*

presents several striking points of similarity. In each case the sporangium consists of an enveloping membrane surrounding a number of discrete spores. Papillae of dehiscence, also, may be induced in *Thraustotheca*; and in *Achlya*, although they are of normal occurrence, may vary greatly, and even become non-functional. Moreover, although the escaping sporangiospores of *Thraustotheca* do not form the hollow sphere characteristic of most species of *Achlya*, they show a degree of adelphotaxy at least comparable to that characterizing *A. paradoxa*, Coker. Furthermore, the sexual organs of *Thraustotheca* very closely resemble those of the *prolifera* group of the genus *Achlya*. These resemblances do not, of course, prove any relationship; but they do seem sufficient to entitle *Thraustotheca* to a tentative systematic position near *Achlya* rather than *Dictyuchus*.

#### SUMMARY.

1. In the processes of sporangium formation and spore liberation *Thraustotheca* agrees with other members of the family. The intersporal substance mentioned by early investigators cannot be demonstrated.
2. *Thraustotheca* is unique among the Saprolegniaceae in that the non-motile sporangiospores escape by bursting the sporangium wall. The fragility of the sporangium wall has been greatly over-emphasized.
3. The sporangiospores may emit motile zoospores, give rise to hyphae of germination, or form dwarf sporangia, according to conditions of the environment.
4. The zoospores are grooved, laterally biciliate, and of a characteristic shape that cannot be adequately described by the terms 'reniform' or 'bean-shaped', that are generally used in this connexion.
5. Gemmae are formed, but represent merely a transient resting state induced by unfavourable environmental conditions.
6. In the development of the sexual structures certain phenomena seem to justify the assumption that the formation of antheridia is dependent on contact of the antheridial filaments with the oogonia; and that oospore formation is, under normal circumstances, definitely correlated with the presence of antheridia on the oogonia.
7. In germination the oospores send out hyphae which either, after limited growth, form sporangia or give rise to extensive mycelia—the type of development depending on the amount of nutrient available.
8. In its structure and development *Thraustotheca* shows a resemblance to *Achlya* that, in the opinion of the writer, is sufficient to entitle it to a systematic position near the latter genus rather than near *Dictyuchus*.

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## EXPLANATION OF PLATES IV AND V.

Illustrating Mr. Weston's paper on the Development of *Thraustotheca*.

The figures were drawn from living material at the level of the stage, with the aid of an Abbé camera lucida. The approximate magnification of the combination of lenses used is given in each case, but applies to the original figures, which have been slightly reduced in reproduction.

### PLATE IV.

- Fig. 1. Young sporangium initial becoming filled with protoplasm.  $\times 550$ .
- Figs. 2-5. Stages in the formation of the septum. Figs. 2-4  $\times 550$ ; Fig. 5  $\times 1,300$ .
- Figs. 6-13. Stages in the development of a small sporangium.  $\times 1,400$ .
- Figs. 14-17. Emergence of a zoospore from a sporangiospore.  $\times 1,400$ .
- Fig. 18. Zoospore on the point of swimming away; side view.  $\times 1,400$ .
- Fig. 19. Cystospore (encysted zoospore) showing discarded cilia.  $\times 1,400$ .
- Figs. 20, 21. Germination of a cystospore.  $\times 1,400$ .
- Fig. 22. Small plant resulting from such a germination in the presence of scanty nutriment.  $\times 550$ .
- Fig. 23. Formation of a dwarf sporangium from a cystospore.  $\times 1,400$ .
- Figs. 24, 25. Germination of a sporangiospore on nutrient agar.  $\times 1,400$ .
- Fig. 26. Three-spored dwarf sporangium formed from a sporangiospore.  $\times 1,400$ .
- Fig. 27. Two successively formed sporangia showing lateral dehiscence and the retention of a large proportion of the spores.  $\times 550$ .
- Fig. 28. Retained sporangiospores, one of which is forming a dwarf sporangium.  $\times 1,400$ .
- Fig. 29. Base of a dehisced sporangium, showing sporangiospores *in situ*, an imprisoned zoospore, a cystospore, and empty sporangiospores.  $\times 1,400$ .
- Fig. 30. Sporangium which still retains a large number of sporangiospores, some of which have emitted zoospores.  $\times 550$ .
- Fig. 31. Same sporangium showing development of germ-tubes when nutriment was added.  $\times 550$ .
- Figs. 32-4. Stages in the development of a bispored dwarf sporangium.  $\times 1,400$ .
- Fig. 35. Portion of a hypha showing endogenous hyphae which have formed sporangia.  $\times 550$ .
- Fig. 36. Abnormal columella-like growth of septum into a sporangium.  $\times 550$ .
- Fig. 37. Portion of a hypha showing thick pectin septum.  $\times 550$ .
- Fig. 38. Sporangium developed at high temperature showing papilla and large central vacuole.  $\times 550$ .
- Fig. 39. Similar sporangium after dehiscence.  $\times 550$ .
- Figs. 40, 41. Incomplete surface cleavage of a punctured sporangium.  $\times 550$ .

### PLATE V.

- Fig. 42. Sporangium formed from a sporangial gemma.  $\times 550$ .
- Fig. 43. Oogonial gemma which has been transformed into a sporangium.  $\times 550$ .
- Fig. 44. Part of oogonium wall, showing pits, after treatment with zinc chlor-iodide.  $\times 1,400$ .
- Fig. 45. Repeated proliferation of oogonial initial with final formation of a sporangium.  $\times 550$ .
- Fig. 46. Formation of oogonium initial and antheridial branch by an oogonial gemma.  $\times 350$ .
- Fig. 47. Irregular hyphal gemma.  $\times 350$ .

Fig. 48. Germination of an oogonial gemma by hyphae.  $\times 550$ .

Fig. 49. Two adjacent oogonium initials; one, with declivous antheridium attached, has formed oospores; the other, without antheridia, has formed a sporangium.  $\times 550$ .

Fig. 50. Formation of oospores in an oogonium which, after proliferating once, has been reached by a declivous antheridial filament.  $\times 550$ .

Fig. 51. Intercalary oogonium showing large pits through which fertilizing tubes have entered.  $\times 550$ .

Fig. 52. Two hyphae of similar origin, one of which formed an antheridium on reaching an oogonium; the other formed a sporangium.  $\times 150$ .

Fig. 53. Androgynous antheridium on an oogonium.  $\times 550$ .

Figs. 54-6. Stages in the development of oospores in an oogonium.  $\times 550$ .

Fig. 57. Later stage in maturation of an oospore.  $\times 1,400$ .

Fig. 58. Mature oospore.  $\times 1,400$ .

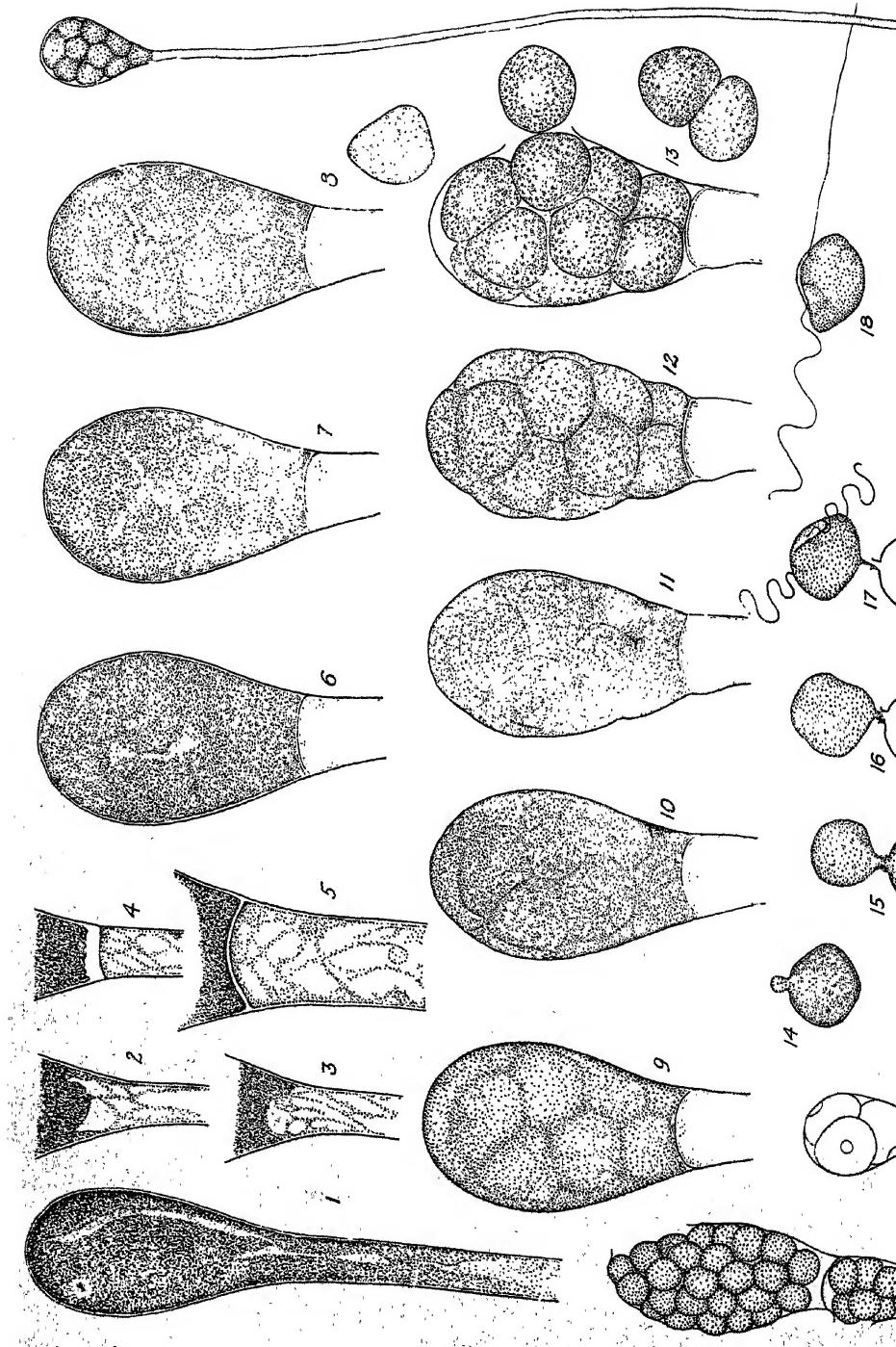
Figs. 59, 60. Changes in oospore preliminary to germination.  $\times 1,400$ .

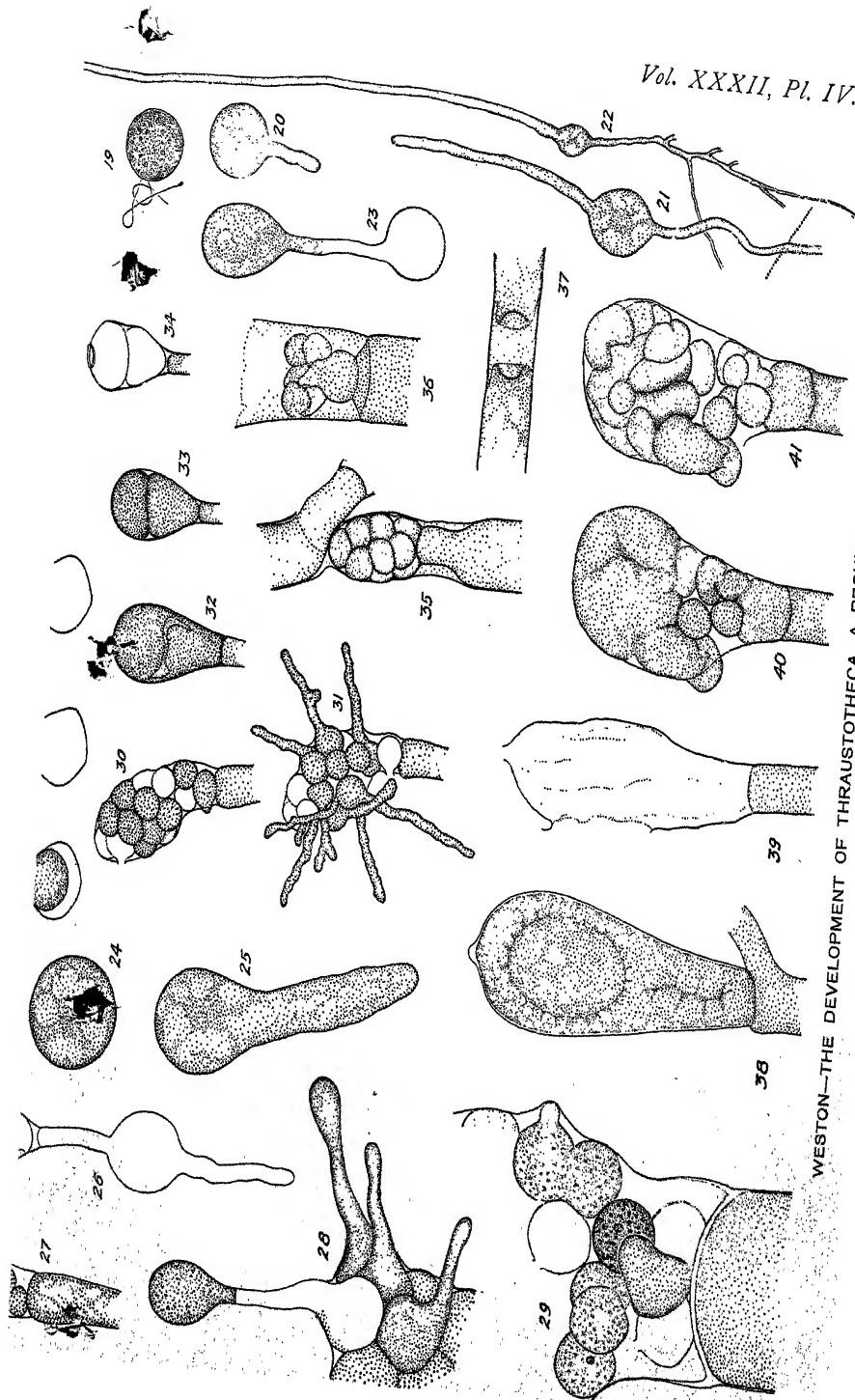
Fig. 61. Formation of germ-tube by free oospore.  $\times 1,400$ .

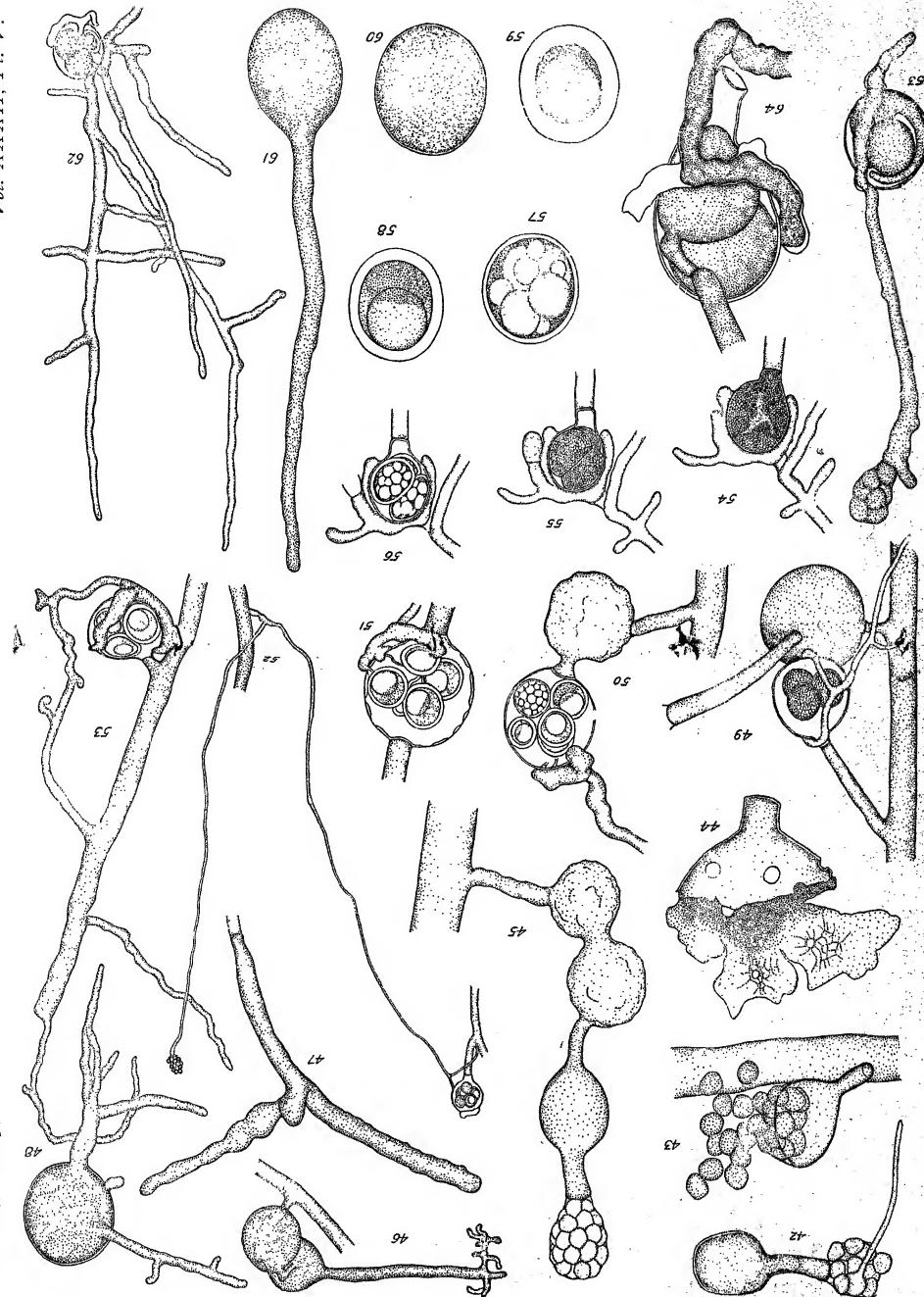
Fig. 62. Formation of a mycelium by oospores germinating *in situ*.  $\times 350$ .

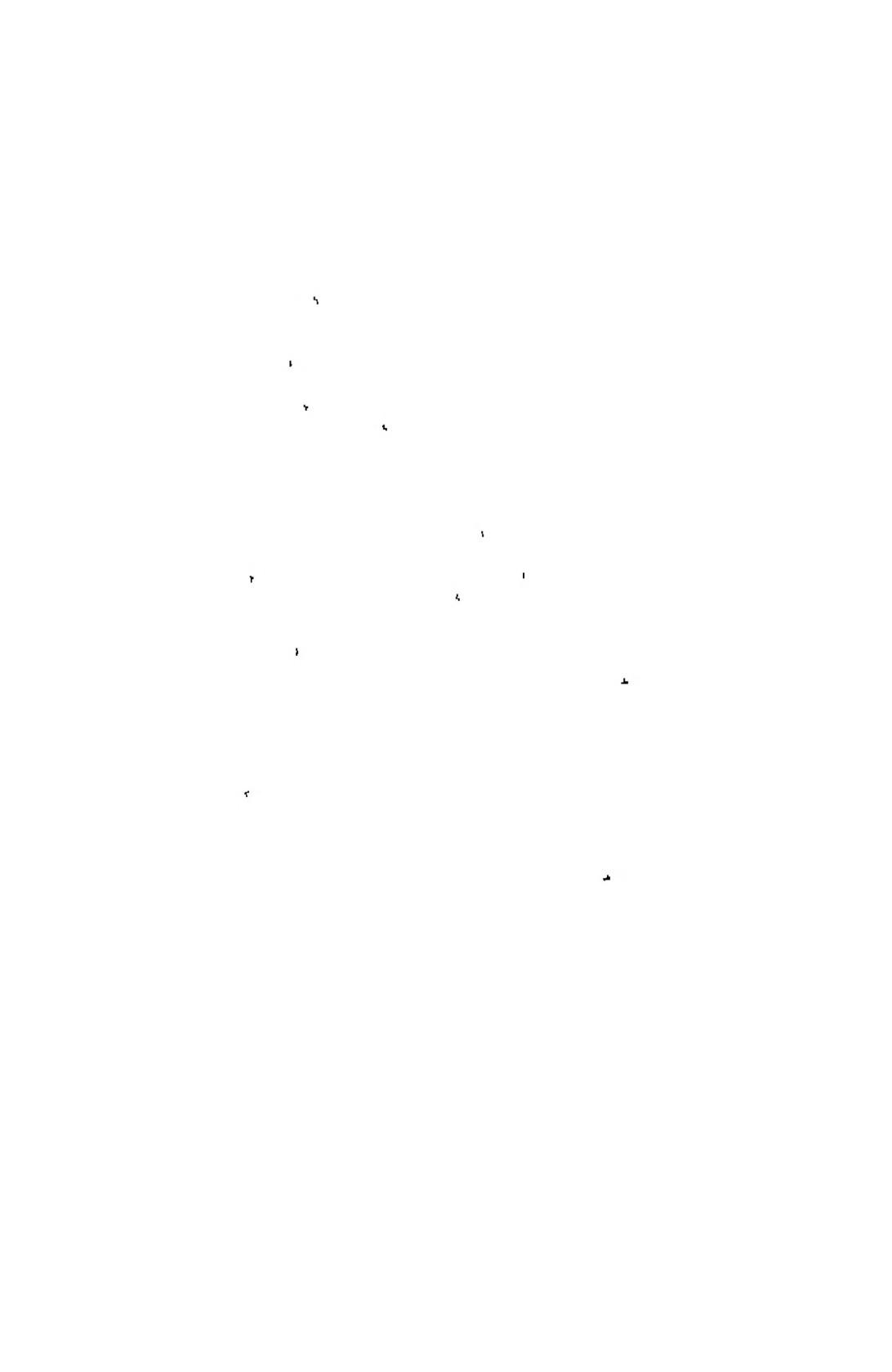
Fig. 63. Formation of a sporangium by an oospore germinating *in situ*.  $\times 550$ .

Fig. 64. Hyphae of germination from oospores *in situ* growing out through the empty antheridia.  $\times 1,400$ .









## NOTE.

**ON A PECULIARITY EXHIBITED BY THE TESTA OF WRINKLED PEAS.**—During a search for a substitute for the porous pot in the construction of a Pfeffer's cell for the demonstration of osmotic pressure the testa of the pea was selected as being likely to afford a non-extensible membrane in which a film of copper ferrocyanide might be deposited. For this purpose peas, soaked twenty-four hours in water until fully swollen, were cut in half, and from the half of the testa which did not contain the micropyle the cotyledons were removed and the testa firmly tied to the end of a glass tube ; this was then filled with copper sulphate solution and the testa immersed in a solution of potassium ferrocyanide.

In place of the expected uniform deposit of copper ferrocyanide over the whole surface of the testa a definite irregular network appeared, the spaces of which remained perfectly colourless. From the character of the network it was at once recognized that wrinkled peas had been employed, and that deposition of the colloid had occurred only at the position of the wrinkles of the dry testa. When round were substituted for wrinkled peas the deposit was perfectly uniform. Clearly, then, there was a difference in permeability in different parts of the wrinkled testa which was not shown in that of the round pea, the depressed parts of the wrinkled pea being quite impervious to the solutions employed, while the wrinkles were freely permeable.

As the wrinkling of the pea testa is a well-recognized Mendelian factor this phenomenon was further investigated. It was found that solutions of sodium chloride had no power to penetrate the parts of the testa which occupied the depressions, and, further, that a solution of safranin stained the soaked testa along the lines of the wrinkles only.

This peculiarity disappeared when the testa was soaked for some hours in warm alcohol, subsequent action of safranin giving a perfectly uniform staining.

The cause of the phenomenon was thus shown to be due to the presence of a waxy bloom on the surface of the testa. This in the case of the round pea easily becomes rubbed off over the whole surface, but in the case of the wrinkled pea persists in those parts which, in virtue of their position in the depressions of the wrinkling, are protected from contact with other peas.

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# The Response of Pilobolus to Light.

BY

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With four Figures in the Text.

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## INTRODUCTION.

MUCH work has been done on the response of organisms to light. Naturally all of the earlier and a large part of the later work was qualitative in nature. Up to the time of Wiesner's classic work on heliotropism, no attempt had been made to express the photic sensibility of plants in quantitative terms. Since the publication of his 'Die heliotropischen Erscheinungen im Pflanzenreiche' (1879), little advance was made along quantitative lines until 1909, when Blaauw published 'Die Perzeption des Lichtes'. In this important contribution modern physical methods are for the first time employed. The conclusions of Blaauw, however, are not in agreement with those of Wiesner, and both contradict without adequate explanation the results of the earlier investigators on phototropism, such as Gardner, Guillemin, Müller, and others.

While considerable progress has been made in the study of the threshold of stimulation—more especially as related to duration and intensity of the light stimulus—we have no complete record of the response of a given organism to carefully graded and measured light energies in the different spectral regions.

### I. THEORIES OF RESPONSE.

A number of theories of response based on the interpretation of data obtained by experimental methods have been formulated, and these shall be referred to as (1) Intensity difference, (2) Ray direction, (3) Wavelength, (4) Energy.

1. De Candolle (1832), the author of the 'intensity' theory, believed that, due to a difference in the light intensity upon the sides of the plant turned toward and away from the source of light, there results an increased carbon dioxide liberation and also an increased transpiration on the lighted side of the organism which brings about an earlier maturation of its cells and hardening of its tissues. Among the adherents to this theory, or to a more modern modification and interpretation of the same, may be mentioned Wiesner (1879), Darwin (1880), Engelmann (1883), Oltmanns (1892), Yerkes (1903), Loeb (1906), and Davenport (1907).

Wiesner attributes the response to the difference in the lighting of the two sides coupled with the inhibiting action of light. He argues that if the organ were entirely transparent and no light were lost by reflection, heliotropic response would be impossible. He offers no experimental proof in support of this view because, as he says, of the difficulty in measuring the difference in light intensity of the two sides.

Charles and Francis Darwin (1880) concluded that the difference in the intensity of the light on opposite sides of the plant modifies the nutation and results in the tropic movement.

Engelmann (1883) found that a very gradual increase, or decrease, of light intensity produced no response in *Paramecium bursaria*. The same difference produced a response if the change took place rapidly. He concluded that response follows a time rate of change of intensity.

Loeb (1906) believes that because of the more intense light on one side of the organism there is produced a difference in chemical constitution of the cells on the two sides which results in heliotropic action.

Oltmanns (1892), by the use of India ink solutions in prismatic wedges, attempted to show that the response of the organism is due to a difference of intensity rather than to direction of ray. A later paper (1897) maintains that the intensity of the stimulus determines the direction, positive or negative, of the response of the organism.

2. Sachs (1876) advanced the theory that the direction and degree of curvature is determined by the direction of the ray passing through the organism. The stimulus is perceived when the long axis of the organ forms an angle with the incident ray.

Strasburger (1878) added substantial proof to the ray-direction theory. By the response of the swarm-spores of *Botrydium* and *Bryopsis* in a trough behind a prismatic wedge filled with a solution of humic acid variously

placed with reference to the light source, he concluded that the directive stimulus is due to the direction of the impinging ray rather than to light intensity. Davenport and Canon (1897) repeated the experiments of Strasburger, using a wedge-shaped container filled with India ink solution, and found that the direction taken by *Daphnia* was in the path of the light rays.

3. The relation between refrangibility and response was first attacked by Payer (1842), who used coloured glass screens spectroscopically tested. He found that cress seedlings behave in red, orange, yellow, and green as in total darkness, but respond positively in blue and violet, the blue being the more active.

Dutrochet (1844), using similar screens, found that cress seedlings failed to respond, but that other seedlings curve towards the red rays. His further experiments lead to the conclusion that response is not due to refrangibility, but to the 'brightness' of the light used.

The Italian botanist, Zantedeschi (1842), showed that *Oxalis multifloris* responds to blue, violet, and green, but not to yellow, orange, and red rays.

D. P. Gardner (1844) studied the effect of the various regions of the sun's spectrum, and concluded that rays of all refrangibility are capable of causing heliotropic response, but that the indigo rays had this property to the highest degree. He decided that the intensity of light had only a subordinate influence, since by increasing the intensity the tropic response increased only slightly.

Guillemin (1858) exposed seedlings of cress and of mustard to the spectral regions obtained by passing the sun's rays through prisms of flint glass, of rock salt, and of quartz. His records show that heliotropic curvature is produced by the invisible chemical and heat rays, as well as by every region of the visible spectrum, as had previously been stated by Dutrochet and Puillet. He further found that the seedlings showed two maxima of response—one in the region between the violet and ultra-violet and the other between the infra-red and green. The positions of these maxima, however, shifted with a change of prisms, or with the position of the sun in the heavens, or with the water vapour in the air. The lower the position of the sun and the greater the amount of water vapour present in the air, the more the second maximum advanced into the visible regions. The more ready response in the violet which Dutrochet obtained, he explained as due to the absorption of the ultra-violet by the lenses used before the prism.

Sachs (1864), using coloured solutions, found heliotropic response only in the blue end of the spectrum. He made no attempt to secure pure colours or to measure the intensity of the light emitted.

Wiesner (1879), by the use of solutions, determined that seedlings of *Vicia* curved in all regions of the visible spectrum excepting in the yellow, which he found to exert a retarding action upon the effect of orange

and red rays when mixed with them. Using the sun's spectrum, he obtained practically the same results. In both series of tests he found a first maximum between violet and ultra-violet, and a second between the red and infra-red. The effect decreased from either end of the spectrum to zero in the yellow.

Dandeno (1903) with glass filters obtained results which differ widely from those of the investigators already mentioned. He found a first maximum in yellow and a second in blue, with the minimum in green. His screens, when spectroscopically tested, did not give pure colour.

Sorokin (1873), Fischer von Waldheim (1872), and Brefeld (1881), studied the effect of light passed through a solution of potassium bichromate, and an ammoniacal solution of copper oxide, on *Pilobolus*, reporting very different results. Thus Sorokin claims that *Pilobolus* fails to grow in light filtered through the solution of copper oxide and that it gives a negative response to light filtered through potassium bichromate. Fischer von Waldheim obtained a strong positive response to the blue light, while Brefeld had a positive reaction in both blue and red, especially strong in the red.

The divergent results of the investigators who have worked towards establishing a relation between refrangibility and response have been summarized by the author in Table I.

TABLE I.

Observer.	Plant.	Infra-red.	Spectral regions.							
			Red.	Orange.	Yellow.	Green.	Blue.	Indigo.	Violet.	
Payer (1842)	Cress	○	○	○	○	○	++	+	+	○
Zantedeschi (1842)	Oxalis	○	○	○	○	+	+	+	+	○
Dutrochet (1844)	Cress	○	○	○	○	+	+	+	+	○
Dutrochet (1844)	Other seedlings	○	+	+	+	+	+	+	+	○
Gardner (1844)	Rape	○	+	+	+	+	+	+	+	○
Guillemin (1858)	Cress	+	++	+	+	+	+	+	+	++
Sachs (1864)	Mustard	○	○	○	○	+	+	+	+	+
Müller (1872)	Cress	+	+	+	+	+	+	+	+	
Sorokin (1873)	<i>Pilobolus</i>	—	—	—	—	○	○	○	○	
Fischer von Waldheim (1875)	<i>Pilobolus</i>	○	○	○	—	+	+	+	+	
Brefeld (1881)	<i>Pilobolus</i>	+	+	+	+	+	+	+	+	
Wiesner (1879)	<i>Vicia</i>	+	+	+	—	+	+	+	++	+
Gräntz (1898)	<i>Pilobolus</i>	+	+	+	+	+	+	+	+	+
Dandeno (1903)	Seedlings	+	+	+	++	+	+	+	+	
Blaauw (1909)	<i>Phycomyces</i>	○	○	○	+	+	+	++	+	+

The above summary shows the conflicting results obtained by a number of investigators on the response of plants to rays of light of different refrangibility.

○ = no response, + a positive, ++ maximum response, — negative response.

4. The attempts to correlate the heliotropic response in plants with an energy value of the light appears first in the work of N. J. C. Müller (1872). While experimenting with cress seedlings in the objective spectrum, he found

that the maximum shifted in repeated experiments, and he concluded that the 'mechanical intensity' varied for one and the same colour. He believed that the blue-violet rays, because of their small intensity, were absorbed in the cells of the lighted side, hindering growth on that side. The longer waves, because of their greater energy, penetrated the tissue more deeply, having equal effect on the two sides, and no curvature resulted. He did not give further experimental proof of this theory.

Haberlandt (1902) developed a very interesting theory of response. He considered the epidermal cells as small lenses which focus the light rays upon the sensitive protoplasmic membranes of the underlying cells. Maxwell and Lebedev showed that light exerts a pressure approximately equivalent to 0.4 mg. to the square metre of absorbing surface, and Haberlandt suggests that the response is due to the pressure exerted by the light in a way somewhat similar to the response of a tendril when stimulated by a pressure equal to a weight of 0.0002 mg.

Wager (1909) modified Haberlandt's 'ocelli' theory in various ways. He believed that the effect of light is due to the absorbed rays in the colouring matter of the cells and not to a mechanical action upon the protoplasmic membranes. He found that the response depends not upon the intensity of the light, but upon its quality, the more refrangible rays being the more active.

Radl (1903) had previously proposed a theory of photic response in animals somewhat similar to that of Haberlandt in plants. He believed that orientation was a direct reaction to light pressure, which, as he says, may resemble the pressure of an air-current.

Nathansohn and Pringsheim (1907) applied to reaction in plants the Talbot law which states that a definite quantity of light energy must be used to produce perceptible reaction. Pringsheim (1912, p. 157) found reaction to vary with phototropic attunement, or degree of adjustment in physiological condition according to the amount of light to which the plant was exposed previous to and during the period of one-sided illumination. In an earlier article (1909, p. 274) he stated that exact measurements in his experiments were impossible, since there was no method of expression for the value of the light source in definite units.

Blaauw (1909), by determining in photometric units the spectral regions, and calculating the energy values for each region from Langley's curve, attempted to explain the lack of harmony in the results of preceding investigators on the basis of energy distribution and of photo-chemical processes. From his experimental data on response of *Avena* and of *Phycomyces* in the spectral regions, he was able to construct curves consistent with those for visual sensitivity, but with the maxima located in the blue and indigo.

Clark (1913) gave remarkable results in his paper on negative photo-

tropism in *Avena sativa*, in which he found that response takes place according to the Talbot law only in the so-called 'first positive' reaction, and that it does not follow the Talbot law in negative or in the 'second positive' reaction. He made no claim to definite values of intensity (p. 739), since he was unable to make exact photometric measurements.

Jennings (1906) and Verworn (1913) attribute response to a change in the physiological state in the organism. The physiological state depends upon metabolic processes which are influenced by external factors.

## II. STATEMENT OF PROBLEM.

From a consideration of the literature it is evident that the chief reason for the unsettled condition in regard to heliotropic response lies in the lack of accurate measurements of the quantity and quality of the light. The writer, by carefully measuring and calibrating the quality and intensity of the light stimulus, undertook the difficult task of correlating, if possible, the conflicting results and views held with reference to the nature of the light stimulus. This was attempted (1) by a study of the response of *Pilobolus* to carefully calibrated light of different wave-lengths and intensities, and (2) by a determination of the energy relation, if any, between this light and heliotropic response.

## III. MATERIALS AND METHODS.

To determine the relative energy values in the different spectral regions it was necessary to employ very delicate physical instruments capable of calibration in standard units. A thermopile and galvanometer constructed by Professor W. W. Coblenz of the United States Bureau of Standards were used for this purpose. The thermopile was chosen because of its non-selective action in the different regions of the spectrum. The spectral regions used for experimental purposes were tested with a spectrometer from Adam Hilger and Co., London, and the limits of the wave-lengths determined. Cultures of *Pilobolus*, grown under controlled conditions and kept in absolute darkness for three hours preceding the formation of the sporangiophores, were exposed to these measured spectral regions, and the presentation time determined in each region. The threshold of stimulation thus found was taken as the measure of the sensitivity of *Pilobolus* to light of the different spectral regions.

The apparatus was of the type quite generally used in spectral experiments where artificial light is the source of illumination (Yerkes, 1910; Laurens, 1911; Day, 1911; Gross, 1913). Two light-tight compartments (*a*, Fig. 1), each 270 × 30 × 30 cm., were placed as wings at angles of 120° to a middle compartment (*b*), 120 × 30 × 30 cm. The inside was blackened throughout with lampblack and provided with screens (*c*) to shut off

all lateral radiation. In each end of the apparatus was a metal box (*d*) with adjustable slit (*e*) in which the light source was enclosed.

At 154 cm. (60·5 in.) from the light source a lens (*i*), having a focal distance of 71·12 cm. (28 in.), was placed. Seventy-nine cm. (31·1 in.) from each lens a carbon bisulphide prism (*P*) deflected a spectrum into the experimental chamber through adjustable slits in a screen (*m*). It will be noted that the spectrum thrown on the screen was at twice the distance from the lens of that reported by Laurens, Day, and Gross. Much better spectral results were thus obtained. The thermopile (*T*) was connected in series with the galvanometer (*G*), and deflexions of the galvanometer were read with telescope and scale at a distance of 1 metre. A tube (*n*), with the inside completely blackened, led from the thermopile to the Hefner

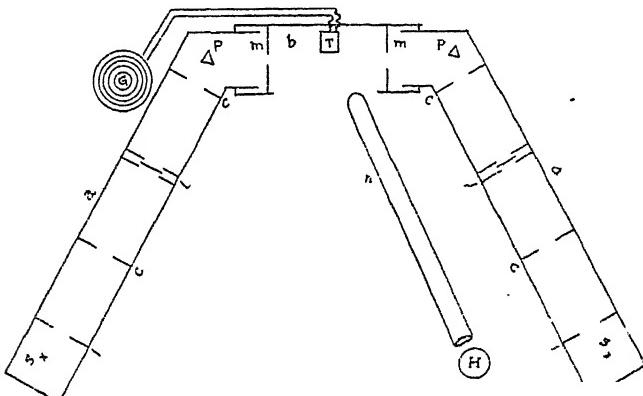


FIG. 1. General plan of apparatus.

lamp (*H*), and served to exclude all lateral radiation and draughts from the thermopile. This apparatus was located in a darkened basement room of the Natural History Building. The walls of this room were blackened, and all lights used in connexion with the apparatus were adjusted so that no stray rays could enter. The temperature and moisture conditions were comparatively constant. The room was thoroughly aired for two hours before each experiment.

The junctions of the thermopile were composed of bismuth and silver welded with tin and covered with platinum-black and lampblack. When a ray of light falls upon this surface at the junction of the two metals it is absorbed as heat and transformed by the junctions into electromotive force. A deflexion of the galvanometer in series with the thermopile indicates the intensity of the radiation. The galvanometer used to indicate the energy absorbed had a moving astatic system suspended by a fine quartz fibre. It was shielded from magnetic disturbance by tubes of soft iron having small apertures through which the scale reflected by the

suspended mirror could be read. Small magnets placed near the galvanometer served to adjust its sensitiveness. The wires connecting the galvanometer to the thermopile were insulated and enclosed in a glass tube, which in turn was covered with tin-foil connected with the earth to prevent disturbances arising from slight variations of room temperature.

Much difficulty was to be anticipated in the manipulation and adjustment of instruments of such delicacy and precision. Although set up on a basement floor of heavy cement, the closing of doors in adjoining rooms or walking in the passage-ways caused tremors that interfered with galvanometer readings. Street cars passing a block away could be detected by a change in the sensitivity of the galvanometer. For these reasons the determinations were made at night and a time chosen when weather conditions were quiet and when very few people were working in the building.

The light sources used were a Nernst lamp of single glower type, obtained from the Westinghouse-Nernst Company, and a 200-watt nitrogen-filled tungsten Mazda made by the General Electric Company. Both were used on a 110-volt alternating current from the University power plant. The voltage was regulated by a volt rectifier and was remarkably constant. Each lamp was adjusted and carefully centred for the lens and prism. The lamp once adjusted remained fixed throughout a series of tests. In the Nernst lamp the glower, being 1 mm. in diameter, served in the place of a narrow slit. A wide slit in front of the glower served to admit the direct light to the lens and to cut off all lateral radiation. The light projected on the prism from the nitrogen-filled tungsten lamp was passed through a slit approximately 5 mm. wide in front of the globe.

The spectral region admitted to the experimental chamber was sufficiently large to cover the slit of the thermopile. Each region used was admitted through a separate slit ( $2\frac{1}{2} \times 10$  mm.) cut in the slides of a photographic plate-holder. The frame for these holders was permanently attached to the base of the apparatus and the slide corresponding to the region to be studied was inserted at the focus of the rays for this region. The thermopile, when measurements were in progress, was placed at 10 cm. from the slit.

The standard of light energy used was a Hefner lamp manufactured by Max Kohl and tested in the German Reichsanstalt. The conditions prescribed for its use were strictly followed, and it was placed at 2 metres from the thermopile, the latter enclosed in the experimental chamber. The energy value of this Hefner lamp at 2 metres distant from the thermocouple was determined in terms of the deflexion of the galvanometer. The energy of the light from any of the spectral regions from the different light sources was similarly read, and expressed in terms of the Hefner lamp reading.

The mechanical value of radiation from the Hefner lamp burning under standard conditions as determined by Ångstrom is  $20.6 \times 10^{-8}$  sec. cm.<sup>2</sup>, or about 8.3 ergs (Nichols, 1905) per second per square centimetre at a distance of 1 metre (cf. Kniep and Minder, 1909). At 2 metres the radiation has one-fourth of this value, or 2.075 ergs. Accordingly, whatever the sensitiveness of the galvanometer, the deflexion produced by the Hefner lamp under the given conditions is equivalent to 2.075 ergs. The energy values of the spectral regions used in the several experiments were measured and expressed as above indicated.

Since a galvanometer of the delicacy of the one used is subject to disturbance, its sensitivity was frequently tested, and if necessary it was readjusted by a slight change in the position of the controlling magnet (cf. Coblenz, 1911). To prevent the air being vitiated by a too frequent use of the Hefner lamp, the red, yellow, and green regions of the spectrum were very accurately determined in their relation to the Hefner lamp standard, and these regions were then used as standards of comparison for other regions of the spectrum. It is evident that any region having its energy value once determined may serve as such a standard. The energy values of the opposite ends of the spectrum, which are so widely different, were thus measured with the galvanometer adjusted in sensitivity to the region in which the experiments were carried on. The values in every case, as shown in Tables IV and V, are expressed in terms of the Hefner lamp values and are directly comparable to each other.

In the earlier work with the galvanometer the average of a series of ten deflexions was taken in each spectral region. It was subsequently found that three or four readings would give practically the same result, and, moreover, there was less chance for error arising from the change of magnetic and temperature conditions.

#### *Pilobolus.*

*Pilobolus* grows abundantly on manure obtained from the University dairy barns. Coemans (1859), Klein (1872), Brefeld (1881), and others have described its culture and mode of growth. The sporangiophores late in the afternoon appear first at right angles to the substratum, due to a negative hydrotropism, and later grow vertical, due to negative geotropism (Pfeffer, 1906). At this stage the sporangiophores are extremely sensitive to light stimuli. In the evening the tips gradually grow into spherical yellow knobs, and the sensitivity of the sporangiophore is very much decreased (Jolivette, 1914). During the night the sporangiophores become distended just below the yellow knobs, and the characteristic transparent bulbs are formed. In the morning the matured sporangia are projected with considerable force.

A number of preliminary experiments were necessary to determine its behaviour under the normal laboratory conditions. It was found that the rapidity of sporangial development bears a direct relation to the temperature. At 28° C. in the greenhouse sporangia are matured and projected early in the morning; at 20° C. they are ejected at noon; and at temperatures maintained below 8° C. they are matured and ejaculated after four days.

It was noted that in direct light there is a tendency to earlier bulb formation than in the shadow, which is probably due to difference in transpiration (Lakon, 1907). Cultures which were well ventilated and kept in complete darkness developed sporangiophores of the usual length and ejected the sporangia but little later in the day than those grown in the same greenhouse in the light. Cultures kept in a laboratory where illuminating gas was used died out when windows were closed (cf. Crocker and Knight, 1908). In this series of experiments special care was taken to work in rooms free of gas.

Cultures of *Pilobolus* were grown in cases in the greenhouse at a relative humidity of 90 per cent. They were transferred early in the afternoon, before any tips were visible, to trays of moist sand and covered with earthen jars furnished with bent-pipe ventilator tubes. Late in the afternoon a culture was taken from the tray, revolved for one minute in a measured spectral region to attune all parts equally (Pringsheim, 1912, p. 159; Clark, 1913, p. 740), and then was exposed to one-sided illumination from this region for a definite period. During the exposure the culture rested on moist sand and was protected with a blackened shield to exclude stray light and to maintain the moisture conditions uniform. The moisture content of the air under the blackened shield as measured with Lambrecht's polymeter ranged between 85 and 92 per cent. relative humidity. The culture after exposure was returned to the tray and covered with the ventilated jar. After one hour it was examined with a reading glass and the number of curved sporangiophores recorded. Only cultures having sporangiophores with pointed tips were used. Strict observance of the physiological state was found necessary since a culture with swollen tips exposed for three hours to the green rays failed to respond, while one with pointed tips responded in seventy minutes. The time of presentation which was required to produce a curvature after a transmission time of one hour in approximately one-half the specimens in a culture, at the period of greatest sensitivity to stimuli, was taken as the standard for the measurement of the reaction of *Pilobolus* to light. No account was taken of reactions which occurred in extremely short intervals of time designated by Clark as 'first positive' curvatures. The longer intervals recorded in the following data without doubt correspond to those which he calls 'second positive' reactions.

## IV. EXPERIMENTAL DATA AND RESULTS.

In Table II the measurements of wave-lengths as determined on a Hilger spectrometer for the Nernst and the nitrogen-filled tungsten Mazda lamps used in these experiments are given.

TABLE II.

Colour.	Nernst.		Tungsten.	
	Included wave-lengths.	Mean wave-length.	Included wave-lengths.	Mean wave-length.
Red	704 712	708	725 691	708
"	695 638	667	692 635	666
Orange	647 616	631	653 610	631
"	650 564	612	640 581	611
Yellow	625 553	589	603 576	589
"	679 551	585	609 558	583
Green	570 510	540	552 525	538
"	543 507	525	542 503	523
Blue	510 482	496	570 486	498
"	479 461	470	490 456	473
Indigo	472 458	465	468 459	464
"	444 432	438	442 431	437
Violet	430 408	414	420 410	415
"	410 385	398	408 388	398

Table III gives the data for the galvanometer deflexions produced by the Hefner lamp at a distance of 2 metres, and for the red region of the Nernst lamp. The energy value of the red region of the Nernst lamp was calculated from the Ångstrom value of the Hefner.

TABLE III.

Light source.	Colour.	Wave-length.	Galvanometer deflection in mm.	Average deflection in mm.	Ergs per sec. per cm. <sup>2</sup>
Hefner lamp		Total radiation at 2 metres	540—70 = 470 530—90 = 440 510—70 = 440 520—70 = 450	450	2.075
Nernst lamp	Red	±667	603—365 = 238 595—350 = 245 600—350 = 250 590—370 = 220	238	1.097

Table IV gives the scale readings for the spectral regions of the Nernst lamp with the values expressed in ergs. The first determinations were made, using the energy value of wave-length  $\pm 667$  equal to 1.097 ergs, found in Table III, as the standard of comparison. The energy value of wave-length  $\pm 540$  equal to 0.344 ergs, as found in this determination, was then taken as the standard for following spectral regions. Another series of measurements is then recorded where the Hesner lamp at 2 metres serves as the direct standard. The results from the data here given are later summarized in Table IX.

TABLE IV.

<i>Light source.</i>	<i>Standard of comparison.</i>	<i>Spectral region.</i>	<i>Galvanometer deflections in mm.</i>	<i>Average deflection in mm.</i>	<i>Ergs per sec. per cm.<sup>2</sup></i>
Nernst lamp	$\pm 667$ (from Table III)	$\pm 667$ (Red)	445—155 = 290 470—195 = 275 470—210 = 260 495—235 = 260	271	1.097
"	$\pm 612$ (Orange)		445—345 = 100 440—360 = 80 485—400 = 85 465—375 = 90	85	0.344
"	$\pm 540$ (Green)		352—315 = 37 373—338 = 35 403—369 = 34 453—409 = 44	37	0.149
"	$\pm 496$ (Blue)		497—475 = 22 523—500 = 23 540—520 = 20 554—524 = 30	24	0.097
"	$\pm 470$ (Blue)		448—430 = 18 468—445 = 23 490—475 = 15 540—522 = 18	19	0.077
Galvanometer sensitivity changed.					
$\pm 540$ used as standard.					
$\pm 540$	$\pm 540$ (Green)		290—235 = 55 300—250 = 50 335—295 = 40 345—305 = 40	46	0.149
"	$\pm 585$ (Yellow)		410—320 = 90 410—315 = 95 415—320 = 95 420—335 = 85	91	0.295
$\pm 540$	$\pm 414$ (Violet)		355—340 = 15 330—318 = 12 340—325 = 15 336—327 = 9	12	0.038
Galvanometer changed in sensitivity.					
Hesner lamp	Ångstrom value	Total radiation at 2 m.	595—255 = 340 520—180 = 340 530—190 = 340 530—190 = 340	340	2.075
Nernst lamp	Hesner value	$\pm 708$ (Red)	545—260 = 285 520—260 = 260 505—240 = 265 500—250 = 250	265	1.616

<i>Light source.</i>	<i>Standard of comparison.</i>	<i>Spectral region.</i>	<i>Galvanometer deflexions in mm.</i>	<i>Average deflexion in mm.</i>	<i>Ergs per sec. per cm.<sup>2</sup></i>
Nernst lamp	Hefner value	$\pm 631$ (Orange)	245 - 140 = 105 230 - 125 = 105 235 - 120 = 115 215 - 115 = 100	106	0.647
"		$\pm 589$ (Yellow)	235 - 190 = 45 240 - 185 = 55 165 - 115 = 50 175 - 125 = 50	50	0.305
"		$\pm 523$ (Green)	242 - 220 = 22 270 - 249 = 21 282 - 261 = 21	21	0.128
"		$\pm 437$ (Indigo)	310 - 302 = 8 240 - 231 = 9 200 - 191 = 9 187 - 178 = 9	9	0.055
$\pm 523$		$\pm 523$ (Green)	255 - 195 = 60 260 - 175 = 85 230 - 155 = 75 300 - 235 = 65	71	0.128
"		$\pm 464$ (Indigo)	375 - 345 = 30 340 - 310 = 30 330 - 285 = 40 330 - 290 = 40	35	0.063

Table V gives the galvanometer deflexions in the spectral regions of a 200-watt nitrogen-filled tungsten Mazda lamp as compared with those of the Hefner—the values being expressed in ergs. Since the spectrum of the tungsten lamp was but 8 mm. in width and the slit of the thermopile 18 mm., a correction was necessary in order to make the readings comparable with those of the wider spectral regions of the Nernst lamp. The calculations for the energy values in red ( $\pm 708$ ) and yellow ( $\pm 589$ ) are made directly from comparison with the energy from the Hefner lamp. Other determinations were made and compared with the energy of the yellow region. These values are later summarized in Table IX.

TABLE V.

<i>Light source.</i>	<i>Standard of comparison.</i>	<i>Spectral region.</i>	<i>Galvanometer deflexions in mm.</i>	<i>Corrected deflection in mm.</i>	<i>Ergs per sec. per cm.<sup>2</sup></i>
Hefner lamp	Ångstrom value	Total radiation at 2 m.	480 - 370 = 110 485 - 360 = 125 490 - 370 = 120 500 - 385 = 115 Average = 117	117	2.075
Tungsten lamp	Hefner value	$\pm 708$ (Red)	550 - 350 = 200 545 - 345 = 200 540 - 340 = 200 540 - 340 = 200 Average = 200	450	7.980
"		$\pm 589$ (Yellow)	504 - 440 = 64 512 - 450 = 62 522 - 460 = 62 522 - 462 = 60 Average = 62	140	2.480

<i>Light source.</i>	<i>Standard of comparison.</i>	<i>Spectral region.</i>	<i>Galvanometer deflections in mm.</i>	<i>Corrected deflection in mm.</i>	<i>Ergs per sec. per cm.<sup>2</sup></i>
Galvanometer changed in sensitivity. $y \pm 589$ used for comparison.					
Tungsten lamp	$\pm 589$	$\pm 589$ (Yellow)	$430 - 310 = 120$ $450 - 330 = 120$ $495 - 355 = 140$ $520 - 380 = 140$ Average = <u>130</u>	292	2.480
"	$\pm 631$	$\pm 631$ (Orange)	$440 - 130 = 310$ $430 - 135 = 295$ $445 - 130 = 315$ $435 - 120 = 305$ Average = <u>305</u>	688	5.843
"	$\pm 589$	$\pm 589$ (Yellow)	Galvanometer changed in sensitivity. $400 - 230 = 170$ $405 - 240 = 165$ $425 - 260 = 165$ $435 - 260 = 175$ Average = <u>169</u>	378	2.480
"	$\pm 667$	$\pm 667$ (Red)	$610 - 225 = 385$ $610 - 215 = 395$ $580 - 190 = 390$ $590 - 195 = 395$ Average = <u>391</u>	879	5.760
"	$\pm 612$	$\pm 612$ (Orange)	$438 - 232 = 206$ $445 - 238 = 207$ $495 - 275 = 220$ Average = <u>211</u>	474	3.109
"	$\pm 523$	$\pm 523$ (Green)	$310 - 225 = 55$ $395 - 315 = 80$ $420 - 345 = 75$ $440 - 365 = 75$ Average = <u>71</u>	162	1.050
"	$\pm 464$	$\pm 464$ (Indigo)	$315 - 278 = 37$ $328 - 290 = 38$ $410 - 372 = 38$ $385 - 340 = 45$ Average = <u>39.5</u>	89	0.578
"	$\pm 437$	$\pm 437$ (Indigo)	$210 - 185 = 25$ $210 - 185 = 25$ $250 - 228 = 22$ $250 - 220 = 30$ Average = <u>25</u>	56	0.364
"	$\pm 398$	$\pm 398$ (Violet)	$440 - 432 = 8$ $488 - 478 = 10$ $508 - 498 = 10$ $508 - 499 = 9$ Average = <u>9</u>	20	0.130
Galvanometer changed in sensitivity.					
"	$\pm 589$	$\pm 589$ (Yellow)	$358 - 128 = 230$ $365 - 140 = 225$ $365 - 145 = 220$ Average = <u>225</u>	506	2.480

Light source.	Standard of comparison.	Spectral region.	Galvanometer deflexion in mm.	Corrected deflexion in mm.	Ergs per sec. per cm. <sup>2</sup>
Tungsten lamp	$\pm 589$	$\pm 540$ (Green)	385-270 = 115 460-365 = 125 465-335 = 130 460-335 = 125 440-320 = 120 Average = 123	277	1.350
"	$\pm 496$ (Blue)		460-410 = 50 460-410 = 50 450-400 = 50 440-390 = 50 Average = 50	112	0.735
			Galvanometer changed in sensitivity.		
"	$\pm 589$ (Yellow)		365-165 = 200 360-140 = 220 385-165 = 220 385-170 = 215 Average = 214	481	2.480
"	$\pm 470$ (Blue)		335-282 = 53 387-328 = 59 400-345 = 55 415-345 = 70 Average = 59	133	0.686

Tables VI and VII give the data for the determination of the presentation periods of *Pilobolus* in the different spectral regions. It was not deemed necessary to repeat here the periods of exposure longer and shorter than the ones which gave the final decisive results.

TABLE VI.

Light source.	Series No.	Colour.	Wave-length.	Temp. °C.	Time of exposure in mins.	% Positive.	% In-different.	Presentation time in min.
Nernst lamp	Rn 1	Red	$\pm 708$	24.6	6.55-7.33 = 78	60	40	-
	Rn 2	"	"	23.1	4.23-5.39 = 76	0	100	
	Rn 3	"	"	24.0	4.51-6.11 = 80	93	7	78
R 11	"	$\pm 667$	24.3	6.13-7.28 = 75	0	100		
R 12	"	"	25.7	4.05-5.21 = 76	88	12		
R 13	"	"	24.0	4.04-5.21 = 77	100	0		76
On 1	Orange	$\pm 631$	24.0	4.36-5.51 = 75	64	46		
On 2	"	"	24.0	5.53-7.05 = 72	0	100		
On 3	"	"	25.0	4.55-6.12 = 77	90	10		75
O 13	"	$\pm 612$	26.3	5.56-7.08 = 72	0	100		
O 16	"	"	24.5	4.18-5.33 = 75	100	0		
O 17	"	"	24.7	7.56-9.09 = 73	52	48		73
Yn 1	Yellow	$\pm 589$	24.7	4.18-5.30 = 72	60	40		
Yn 2	"	"	24.7	5.12-6.22 = 70	20	80		
Yn 3	"	"	25.0	4.58-6.12 = 74	90	10		72
V 19	"	$\pm 585$	24.8	7.55-9.05 = 70	0	100		
V 20	"	"	24.8	6.01-7.15 = 74	100	0		
V 26	"	"	24.4	7.06-8.18 = 72	50	50		72
G 8	Green	$\pm 540$	24.6	7.50-9.00 = 70	100	0		
G 10	"	"	26.8	6.51-7.56 = 65	0	100		
G 11	"	"	24.3	5.34-6.42 = 68	33	66		69

Light source.	Series No.	Colour.	Wave-length.	Temp. °C.	Time of exposure in mins.	% Positive.	% Indifferent.	Presentation time in min.
Nernst lamp	Gn 1	Green	± 523	24·5	7.02-8.09½ = 67½	62	38	
	Gn 2	"	"	24·0	4.17-5.26 = 60	100	0	
	Gn 3	"	"	23·6	5.04-6.10 = 66	8	92	67½
B 10	Blue	± 496		23·6	7.40-8.40 = 60	0	100	
B 17	"	"		23·7	8.15-9.22 = 67	90	10	
B 19	"	"		24·8	5.41-6.46 = 66	66	34	65
Bn 12	"	± 470		26·0	5.00-6.05 = 65	75	25	
Bn 13	"	"		26·0	6.58-8.00 = 62	25	75	
Bn 16	"	"		24·7	6.48-7.51 = 63	20	80	63
I 2	Indigo	± 464		23·8	4.38-5.40 = 62	55	45	
I 3	"	"		23·2	5.42-6.42 = 60	34	66	
I 4	"	"		23·4	4.24-5.28 = 64	100	0	62
In 9	"	± 437		24·7	5.34-6.31 = 57	0	100	
In 10	"	"		24·6	6.32-7.32 = 60	42	58	
In 11	"	"		23·6	5.54-6.56 = 62	90	10	60
V 10	Violet	± 414		25·7	4.58-5.50 = 52	25	75	
V 12	"	"		24·3	4.21-5.19 = 58	75	25	
V 13	"	"		25·2	6.48-7.44 = 56	60	40	56
Vn 7	"	± 398		25·6	4.49-5.43 = 54	11	89	
Vn 10	"	"		26·5	5.05-6.00 = 55	54	46	
Vn 11	"	"		26·5	6.05-7.01 = 56	74	26	55

TABLE VII.

Light source.	Series No.	Colour.	Wave-length.	Temp. °C.	Time of exposure in mins.	% Positive.	% Indifferent.	Presentation time in min.
Tungsten lamp	Rt 2	Red	± 708	26·6	6.32-7.37 = 65	0	100	
	Rt 3	"	"	24·0	6.39-7.47 = 68	50	50	
	Rt 4	"	"	26·4	4.27-5.36 = 69	71	29	68
Ra 6	"	± 667		23·4	5.36-6.42½ = 66½	57	43	
Ra 7	"	"		23·0	6.46-7.50 = 64	15	85	
Ra 8	"	"		25·6	4.48-5.56 = 68	95	5	66½
Ot 3	Orange	± 631		25·8	4.58-6.02½ = 64½	50	50	
Ot 4	"	"		25·8	6.04-7.10 = 66	70	30	
Ot 5	"	"		25·9	7.12-8.14 = 62	36	64	64½
Oa 1	"	± 612		24·6	6.17-7.21 = 64	50	50	
Oa 2	"	"		23·1	4.20-5.26 = 66	100	0	
Oa 3	"	"		23·2	5.27-6.29 = 62	25	75	64
Yt 4	Yellow	± 589		23·6	6.00-7.01 = 61	0	100	
Yt 8	"	"		25·0	6.05-7.09 = 64	75	25	
Yt 9	"	"		24·4	7.11-8.14 = 63	75	25	63
Ya 12	"	± 585		24·8	6.12-7.15 = 63	42	58	
Ya 13	"	"		25·2	4.54-5.59 = 65	90	10	
Ya 14	"	"		25·7	5.50-6.52 = 62	10	90	63
Ga 1	Green	± 540		21·5	6.33-7.33 = 60	45	55	
Ga 2	"	"		21·5	7.34-8.32 = 58	37	63	
Ga 3	"	"		24·0	4.24-5.26 = 62	100	0	60
Gt 3	"	± 523		26·0	6.54-7.57 = 63	71	29	
Gt 4	"	"		24·9	7.06-8.04 = 58	0	100	
Gt 8	"	"		24·6	5.44-6.44 = 60	82	18	59
Ba 7	Blue	± 496		23·6	5.06-6.05 = 59	67	34	
Ba 8	"	"		24·0	6.15-7.11 = 56	20	80	
Ba 11	"	"		26·0	4.35-5.32 = 57	56	44	57

Light source.	Series No.	Colour.	Wave-length.	Temp. °C.	Time of exposure in min.	% Positive.	% Indifferent.	Presentation time in min.
Tungsten lamp	Bt 9	Blue	$\pm 470$	24·7	5·37-6·30 = 53	20	80	
	Bt 10	"	23·6	4·46-5·41 $\frac{1}{2}$	= 55 $\frac{1}{2}$	94	6	
	Bt 11	"	23·5	7·00-7·54	= 54	37	63	55
	It 6	Indigo	$\pm 464$	24·3	5·45-6·43 = 58	66	33	
	It 7	"	24·8	4·38-5·33	= 55	60	40	
	It 8	"	25·2	6·45-7·35	= 50	25	75	55
	Ia 3	"	$\pm 437$	24·9	6·34-7·24 = 50	0	100	
	Ia 9	"	25·2	7·09-8·03	= 54	32	68	
	Ia 10	"	24·0	4·45-5·42	= 57	84	16	54
	Va 1	Violet	$\pm 414$	25·2	5·00-5·53 = 53	92	8	
	Va 5	"	25·1	5·21-6·12	= 51	50	50	
	Va 6	"	25·3	6·12-7·02	= 50	0	100	51
	Vt 12	"	$\pm 398$	25·2	3·59-4·58 = 59	100	0	
	Vt 13	"	23·5	5·05-5·52	= 52	25	75	
	Vt 14	"	23·8	5·37-6·27	= 50	33	66	50

In Fig. 2 the spectral energy curves for the Nernst and for the tungsten lights represent in graphic form the data recorded in Tables II, IV, and V. Wave-frequencies of light are represented by the ordinates and the mechanical energy in ergs per second by the abscissae. A comparison of the above curves with those of Coblenz (1911) for the Nernst and the tungsten lights, and those of Moll (1907) for the Nernst light, will show that a higher value was obtained in the violet. Although repeated attempts were made to bring these results in the violet into conformity with those of Coblenz and Moll, they were not successful. The difference may be in part due to the use of the Nernst lamp with the globe removed.

A number of investigators have maintained that the response of organisms to light of different nature may be correlated with energy equivalence. *Pilobolus*, exposed to the spectral regions of the Nernst and tungsten lamps differing rather widely in energy, will respond as indicated in Fig. 3. In plotting these graphs the wave-lengths and frequencies are disregarded. The abscissae represent the presentation time and the ordinates the energy of the region expressed in ergs, the data being taken from Tables IV, V, VI, and VII.

An inspection of Fig. 3 shows that an actual decrease in the rate of response takes place with increase of the photic energy. This shows, conclusively, there is no direct relation between response and the energy of the different regions. Thus, the lens theory of Haberlandt (1905) and the orientation theory of Rädl (1903), that response is due to a pressure that the light exerts on the cells of the organ, are not applicable to the response of *Pilobolus*.

Likewise, a statement in Blaauw's theory of response (1909, p. 30), namely, that the plant perceives only the quantity of energy as a stimulus, cannot be taken literally, for he says: 'Für diesen konstanten Effekt ist eine konstante Quantität Energie nötig und es ist also für die Pflanze gleichgültig, wie diese Energie, über Zeit und Intensität verteilt, zugeschürt wird. Die Pflanze empfindet nur die Quantität Energie als Reiz; die

Zeit und die Intensität sind nichts mehr als Faktoren von der Energiemasse. Nur diese Quantität Energie wirkt als Reiz, für die Pflanze selbst besteht weder die Intensität, noch die Zeit als eine absonderliche Grösse.' Fröschel (1909, p. 422) expresses the same view in different words, thus: 'Gleiche

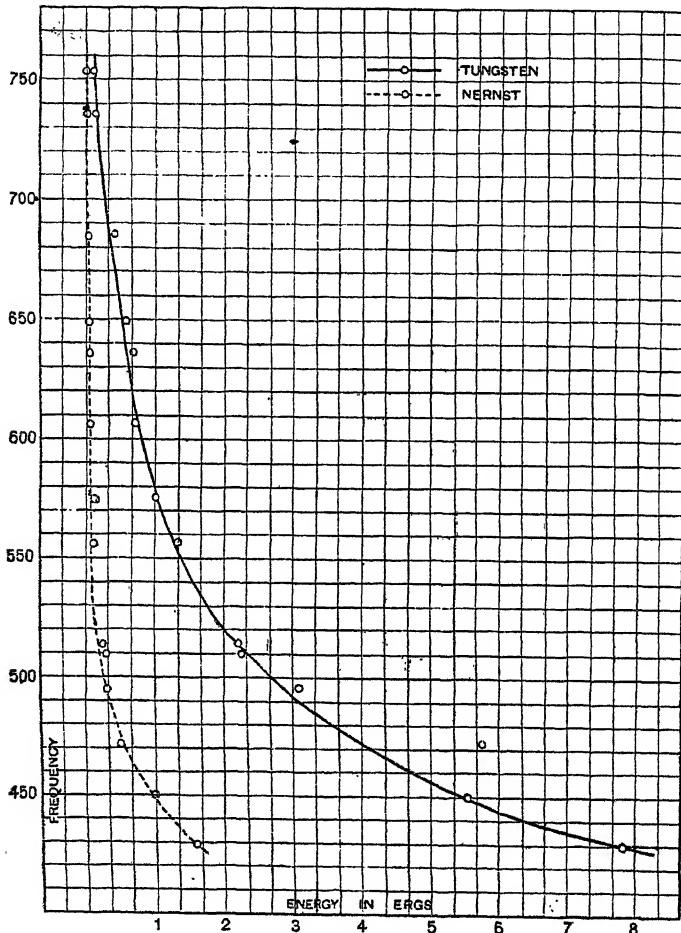


FIG. 2. Spectral energy graphs (from data in Tables II, IV, and V) for the single glower Nernst lamp and the 200-watt nitrogen-filled tungsten Mazda lamp used in these experiments. Ordinates represent wave-frequencies of light and abscissae the mechanical energy in ergs per second per square centimetre.

Energiemengen rufen bei Pflanzen gleiche Reaktionen, beim menschlichen Auge gleiche Empfindungen hervor.'

A superficial study of these curves, however, might lead one to the erroneous belief that increase of energy has an inhibiting effect on the tropic response. If this were true, the decrease in the irritability in the tungsten light towards the higher energy values would be much more rapid

than in those of the Nernst. Further discussion of the energy relation will be taken up later.

If the presentation periods be plotted with reference to frequency of the light waves in the spectral regions to which *Pilobolus* was exposed, the results will appear as in Fig. 4. These graphs are based on the data given

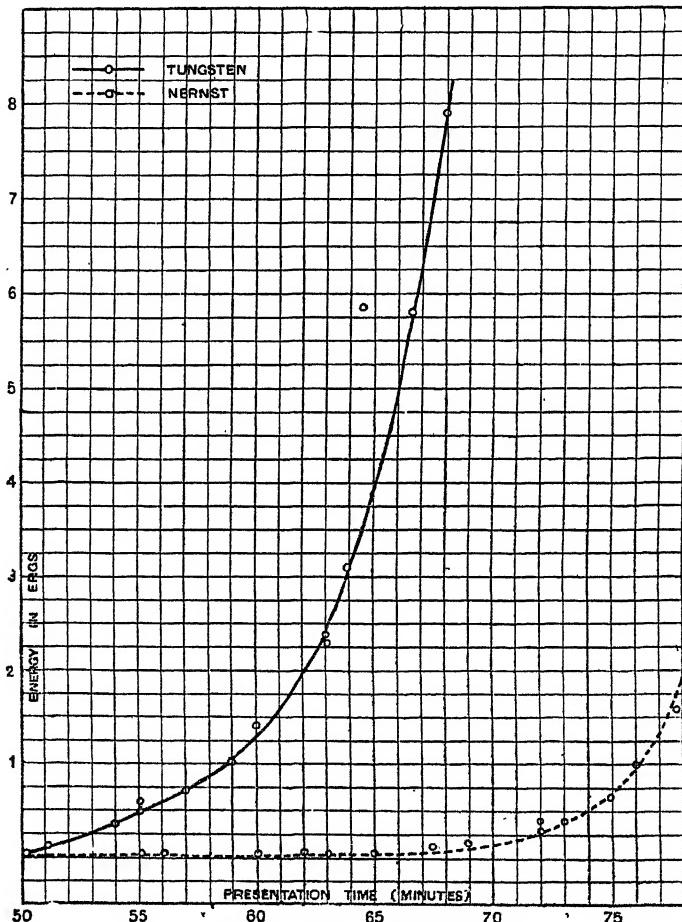


FIG. 3. Graphs (from data in Tables IV, V, VI, and VII) in which abscissae represent the presentation time of *Pilobolus* and ordinates the energy values of the spectral regions to which the plant was exposed.

in Tables II, VI, and VII. In this instance it will be noted that response in *Pilobolus* takes place in every region of the spectrum, and that the presentation period decreases from red to violet, or conversely, that the irritability increases from red to violet. These results then confirm those obtained by Brefeld (1881, p. 60) and Gräntz (1898), and are contrary to those of Sorokin (1874) and of Fischer von Waldheim (1875).

The graphs as given in Fig. 4 are remarkably constant in their gradual

increase from red to violet. At no time was there found in this series of experiments any intermediate minimum such as Wiesner obtained in yellow (1879) and Dandeno in green (1903), nor a maximum, as reported by Guillemin (1857) in the violet and in the red, by Dandeno in yellow, and by Blaauw (1909) in indigo.

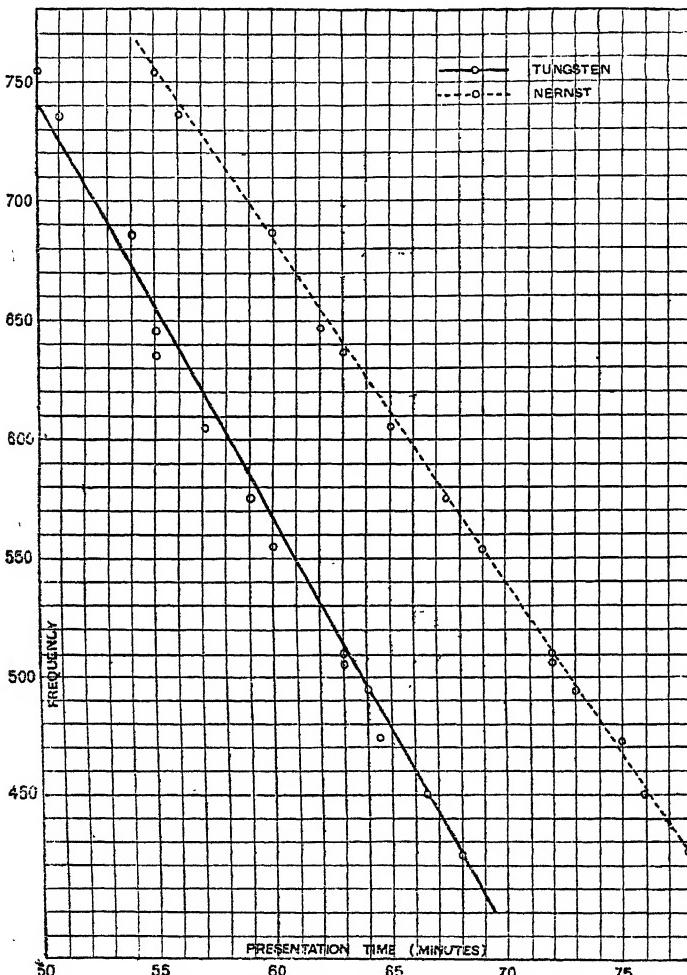


FIG. 4. Graphs (from data in Tables II, VI, and VII) showing presentation times on *Pilobolus* in relation to frequency of light waves. Abscissae represent the presentation times in minutes, and ordinates the frequency of the light waves.

The slope of the graphs from the violet towards the red shows that the response of the organism has a very marked relation to wave-frequency. Calculation of this relation shows that the product of the square root of the frequencies and the presentation time is so nearly uniform as to warrant attention later (cf. Nernst, 1899; Verworn, 1913). The results of this calculation are given in Table VIII.

Attention must be called to the fact that the graphs representing the time and frequency relation (Fig. 4) of *Pilobolus* for the two sources of light (Nernst and tungsten) are not nearly identical, as would be the case were wave-frequencies alone responsible for the differences in the induction periods. Furthermore, the two graphs diverge somewhat from the violet towards the red, giving additional evidence that energy is a factor in the relative time required for phototropic excitation. The fact that energy does play a part in response is further manifest from the results given in Table VIII. This table shows that while the square roots of the frequencies constitute an ascending series, the square roots of the presentation times form a slightly descending series. It should also be noted that the constant for the Nernst is considerably larger than that for the tungsten.

TABLE VIII.

Colour.	Wave-length.	Frequency.	$\sqrt{f}$ .	Nernst. Presentation time in min.	$\sqrt{f} \times t$ .	Trungsten. Presentation time in min.	$\sqrt{f} \times t$ .
Red	± 708	± 424	20·6	78	1606	68	1400
	± 667	± 450	21·2	76	1611	66·5	1409
Orange	± 631	± 473	21·7	75	1627	64·5	1399
	± 612	± 494	22·2	73	1620	64	1420
Yellow	± 589	± 509	22·5	78	1620	63	1417
	± 585	± 512	22·6	72	1627	63	1423
Green	± 540	± 556	23·6	69	1628	60	1416
	± 523	± 574	23·9	67·5	1603	59	1410
Blue	± 496	± 607	24·6	65	1509	57	1402
	± 470	± 638	25·2	63	1587	55	1386
Indigo	± 464	± 648	25·4	62	1574	55	1397
	± 437	± 686	26·3	60	1578	54	1393
Violet	± 414	± 738	27·1	56	1517	51	1383
	± 398	± 753	27·4	55	1507	50	1370

Since the time of response of *Pilobolus* to the light of the tungsten and Nernst lamps, respectively, is by no means in direct proportion to their spectral energy values, and since there is found to exist some relation of the energy to the time of response, the question arises as to the possibility of expressing this relation in simple mathematical terms.

Wiesner (1879) has shown that the excitation of the plant increases less rapidly than the photic stimulus which produces it.

Pfeffer (1883), through the study of the reaction of swarm spores and bacteria to chemical stimuli, established the application of the Weber-Fechner law to plants. This law states that whereas the intensity of the stimulus increases in geometrical progression, the intensity of the reaction increases in arithmetical progression, or that response varies with the logarithm of the stimulus. This is expressed in the formula:

$S_1 - S_2 = A \log \frac{I_1}{I_2}$ , where  $S_1$  and  $S_2$  are the sensations,  $I_1$  and  $I_2$  the respective intensities of the stimuli, and  $A$  is a constant which varies with the quality of the stimulus.

This law, which was developed from a psycho-physical basis by Weber and Fechner (1882), was later shown by Müller (1878) to have a physiological significance. While Pfeffer showed its application to chemotropic stimuli, he expressed a belief that it would be found true in other forms of reaction, such as phototropism and geotropism.

Massart (1888) has shown that the irritability of the sporangiophores of Phycomycetes between two unequal intensities of light follow the Weber-Fechner law in their reaction.

If for the term 'sensation', which belongs to the realm of psychology, we substitute 'irritability' (cf. Preyer, 1874), the Weber-Fechner law may be tested in its application to the present problem within the limits of the measured intensities of the spectral regions of the two light sources. In Table IX are given the measured values of the energy and the presentation time, together with the solution of the formula to obtain the value of constant  $A$  for each given region. The value of  $A$ , however, is seen to decrease rather uniformly through the spectral regions, and this strengthens the belief already expressed, namely that the two factors, wave-frequency and energy combined, give the phototropic response. If the value of  $A$  in each case is multiplied by the square root of the wave-frequency of the region in which the measurements were made, an approximation to a general constant for the whole visible spectral region is obtained as shown in the last column of Table IX.

A comparison of the present series of results with those conforming to the Talbot law, as found by Nathansohn and Pringsheim (1907), Blaauw (1909), and others, shows a considerable difference. If quality of light is an essential, as shown by the present experiments, Pringsheim's methods in the light of these results are not quite fair, since the smoked glass used to reduce the quantity of light would interfere with its quality. This would cut down the different wave-lengths unequally and the heliotropic efficiency accordingly would be indefinite.

Patten working with blow-fly larvae in their relation to different intensities of light found in one series of experiments an excellent agreement with the Weber-Fechner law. A second series of measurements, however, gave different results. He concludes that the conformity with Weber's law, where it does occur, is entirely accidental. The results of the present series of experiments as shown in Table IX do not corroborate Patten's conclusion. Blackman (1905) has shown that a slight change in temperature causes a corresponding change throughout the physiological system of the organism. It is not strange, then, considering the difficulties in obtaining identical experimental conditions, that curves of response should vary considerably. The valuable mass of evidence presented by such investigators as Pfeffer (1883), Massart (1888), and others must lead to the belief that some fundamental factor exists in the protoplasm which gives rise to results conforming to the Weber-Fechner law.

Tröndle (1910) in his effort to bring the results of his work concerning the relation of permeability to light intensity into conformity with the Talbot law has developed a formula deserving attention. His data show that the product of the reaction time less a constant ( $k$ ) multiplied by the intensity is always the same, expressed mathematically thus:

$$I_1(t_1 - k) = I_2(t_2 - k).$$

The present series of data tested by this formula is given in Table X, where  $I_n$  and  $I_t$  = intensity for a given wave-length,  $t_n$  and  $t_T$  = presentation times for 'second positive' reaction in the same wave-length from the Nernst and the tungsten lamps respectively. Here, too, there is seen to be a gradual decrease in the constant  $k$  from red to violet. Again, multiplying the variable constant in each case by the square root of the frequency, a very fair constant result is obtained, as shown in the last column.

TABLE IX.

Wave-length.	Fre-quency.	Energy in ergs per sec. per cm. <sup>2</sup>		Presentation time in min.		$S_1 - S_2 = A \log \frac{I_1}{I_2}$		$I_1$	$S_n - S_t$	$A$	$\sqrt{f}$	$A \sqrt{f}$
		$I_n$	$I_t$	$S_n$	$S_t$	$\log_{10} I_n$	$\log_{10} I_t$					
708	424	1.616	7.980	78	68	0.2084	0.9020	0.6936	10	14.41	20.6	297
667	450	1.097	5.760	76	66.5	0.3020	0.704	0.7302	9.5	13.01	21.2	276
631	473	0.647	5.843	75	64.5	1.8109	0.7666	0.9557	9.5	9.93	21.7	215
612	494	0.344	3.109	73	64	1.5366	0.4926	0.9500	9	9.41	22.2	209
589	509	0.305	2.480	72	63	1.4843	0.3945	0.9102	9	9.88	22.5	222
585	512	0.295	2.360	72	63	1.4098	0.3729	0.9031	9	9.96	22.6	225
540	556	0.149	1.350	69	60	1.1732	0.1303	0.9571	9	9.40	23.6	213
523	574	0.128	1.050	67.5	59	1.1072	0.0212	0.9140	8.5	9.30	23.9	222
496	607	0.097	0.735	65	57	2.0868	1.8629	0.8761	8	9.13	24.6	224
470	638	0.077	0.686	63	55	2.0885	1.8363	0.9498	8	8.42	25.2	212
464	648	0.063	0.578	62	55	2.7993	1.7622	0.9629	7	7.27	25.4	185
437	686	0.056	0.304	60	54	2.7482	1.5611	0.8129	6	7.38	26.3	194
414	738	* 0.038	0.145	56	51	2.5797	1.1613	0.5816	5	8.59	27.1	232
398	753	0.032	0.130	55	50	2.5052	1.1139	0.6087	5	8.21	27.4	225

\* This value is high according to Coblentz.

TABLE X.

Wave-length.	Fre-quency.	Energy in ergs per sec. per cm. <sup>2</sup>		Presentation time in min.		$I_n(t_n - k) = I_t(t_T - k)$		$K$	$K \sqrt{f}$
		$f$	$I_n$	$I_t$	$t_n$	$t_T$	$I_n(t_n - k) = I_t(t_T - k)$		
708	424	20.6	1.616	7.980	78	68	1.616 (78.0 - $k$ ) = 7.988 (68.0 - $k$ )	65.4	1347
667	450	21.2	1.097	5.760	76	66.5	1.097 (76.0 - $k$ ) = 6.514 (66.5 - $k$ )	65.5	1389
631	473	21.7	0.647	5.843	75	64.5	0.647 (75.0 - $k$ ) = 5.843 (64.5 - $k$ )	63.2	1371
612	494	22.2	0.344	3.109	73	64	0.344 (73.0 - $k$ ) = 3.473 (64.0 - $k$ )	63.0	1398
589	509	22.5	0.305	2.480	72	63	0.305 (72.0 - $k$ ) = 2.480 (63.0 - $k$ )	62.2	1399
585	522	22.6	0.295	2.360	72	63	0.295 (72.0 - $k$ ) = 2.360 (63.0 - $k$ )	61.7	1394
540	556	23.6	0.149	1.350	69	60	0.149 (69.0 - $k$ ) = 1.350 (60.0 - $k$ )	58.7	1385
523	574	23.9	0.128	1.050	67.5	59	0.128 (67.5 - $k$ ) = 1.050 (59.0 - $k$ )	58.9	1407
496	607	24.6	0.097	0.735	65	57	0.097 (65.0 - $k$ ) = 0.644 (57.0 - $k$ )	55.5	1365
470	638	25.2	0.077	0.686	63	55	0.077 (63.0 - $k$ ) = 0.686 (55.0 - $k$ )	55.6	1401
464	648	25.4	0.063	0.578	62	55	0.063 (62.0 - $k$ ) = 0.578 (55.0 - $k$ )	54.1	1374
437	686	26.3	0.056	0.304	60	54	0.056 (60.0 - $k$ ) = 0.304 (54.0 - $k$ )	52.9	1391
414	738	27.1	0.038	0.145	56	51	0.038 (56.0 - $k$ ) = 0.145 (51.0 - $k$ )	49.2	1333
398	753	27.4	0.032	0.130	55	50	0.032 (55.0 - $k$ ) = 0.130 (50.0 - $k$ )	48.3	1323

## V. CONCLUSIONS AND THEORETICAL DISCUSSION.

In considering the lack of conformity in the results of the investigations in the field of phototropism, it must be remembered that the plant is an organism existing in a physiological state continuously changing with the varied physical and chemical factors of its environment. A slight change in some one of the factors may markedly change the relation of the organism to every other factor (cf. Verworn, 1913). Attention has already been called to the disturbing influence of the slightest trace of illuminating gas in the laboratory air upon *Pilobolus*. The researches of Crocker and Knight (1908) have shown its vitiating influence upon the higher plants. A review of many articles upon phototropism shows that illuminating gas has furnished the source of light for the experimental work. Thus Wiesner (1879), Figidor (1893), Pringsheim (1909), in order to subject the plant to different intensities of light varied the distance from the gas-flame from a few centimetres to several metres. Even though the presence of the gas in the room was not destructive to the plants used by these investigators, one might see from the work of Richter (1906), and Crocker and Knight (1908), the inhibiting effect that mere traces of the gas, or the products of combustion, have upon the sensitivity. The presence of these deleterious agents unquestionably affects in degrees, according to the distance of removal from the burner, the physiological condition of the plants and thereby effect a change in their irritability. The above objections naturally do not affect those experiments of Wiesner, Figidor, and Blaauw, where the sun or arc lights were used.

A noticeable difference between the results of the present series of experiments and those of earlier workers lies in the absence of the maximal and minimal points of response in the spectrum. Inquiry into the cause for this difference naturally leads first to the study of the nature of the light obtained from different sources.

The theory of light as formulated by Maxwell and accepted by physicists of the present time states that light consists of short electromagnetic waves which are produced by violent agitation of particles either from the electric current or other source of heat. Every source of light used for illumination has its own characteristic spectrum which differs from that of every other source, and the energy of radiation of each wave-length derived from one source differs from that of the corresponding wave-length from every other source (Nutting, pp. 12 and 197; Fery, 1908; Coblenz, 1911). Moreover, the spectrum from any given source changes according as the absolute temperature of the source is increased. The maximal spectral energy is found to move towards the violet end with a rise in the absolute temperature (Ives, 1910; Drysdale, 1908; Nichols, 1903; Wien, 1893). Thus we find that bodies heated to 500°C. emit only red rays in the visible spectrum,

while the sun, at a probable temperature of 6,000° C., emits the greatest amount of energy in the violet (cf. Duff, p. 455).

The Nernst and the tungsten lamps, heated to about 2,300° C. (Hyde), show the spectral energy to increase from the ultra-violet to a maximum in the infra-red (Moll, 1907; Coblenz, 1911). Since we find the energy highest in the red of the visible spectrum, we should expect from the discussion already given that in the response of *Pilobolus*, the slowness of the waves in the red region is in a measure compensated by their greater energy. The frequency having a much more noticeable effect than the energy values of the waves in the response of *Pilobolus* (Table IX, Figs. 3 and 4) may more than compensate for the lack of energy in the violet region. The regions between the red and violet having a gradual decrease in energy and an increase in frequency produce a response intermediate in time between that of the red and the violet. From the physical basis one could then predict the first maximum in ultra-violet and a second maximum in the orange in the response of plants exposed to a light having its maximum spectral energy in the orange, as the energy of this region would more than compensate for the slowness of the waves and a second maximum thus appear.

The results obtained by Guillemin (1857), who by the use of different prisms was able to obtain spectra from the sun in which the relative intensity varied for the same wave-length, substantiate this view. With a rock-salt prism, which transmits dark heat rays, he obtained the first maximum in the ultra-violet and a second maximum in the ultra-red; with a quartz prism, which transmits the chemical rays, he obtained the first maximum in the violet and a second between the red and infra-red; with a flint-glass prism, which best transmits the intermediate rays, he obtained the first maximum in the violet and a second in the green. He further found that the second maximum advanced more and more into the visible spectrum as the water vapour in the air was increased and the position of the sun approached the horizon.

Wiesner's results with seedlings in the sun's spectrum may in a measure be correlated with this line of thought. Due to the vapour, &c., present in the atmosphere, the maximum energy of the sun's spectrum at noon is usually near the yellow (Langley, 1883, p. 33). The use of a biconvex lens would so focus these rays as to bring the intensity in the yellow to a very high value which may be responsible for the indifferent, or even negative heliotropic response (Oltmanns, 1892; Pringsheim, 1907; Blaauw, 1909; Clark, 1913). His results show a very regular decrease in the presentation time from the green into the ultra-violet, and bring the maximum irritability into the ultra-violet region. This would naturally follow from the increasing frequency and very considerable energy of this region, in the same manner as determined and recorded in this paper (Table IX).

In Blaauw's experiments (1909), because of the higher absolute temperatures of the light sources (sun and arc-lamp), the maximum energy is near the yellow. He obtained no response in the red end beyond the yellow-orange. In the indigo with a higher frequency and lower energy value he found a maximum response. This again accords beautifully with the results already described in this paper.

Divergent views held by previous investigators regarding the region of maximum response in the spectrum can be readily explained on the basis of energy value and frequency. That this has not previously been done is largely to be attributed to the difficulty encountered in determining the spectral values. Thus, Gardner (1844) observed that the intensity of light had only a subordinate influence. Sachs (1867) and Loeb (1906) state that the shorter waves are the more active and that the reaction is proportional to the intensity. Towle (1900) says of *Cypredopsis* that the response is shorter in the stronger light, 'though the difference is too slight to warrant one in drawing any inferences from it'. Allen and Jolivette (1913, p. 581) concluded from their experiments where coloured glass screens were used that 'the light of short wave-length has no preponderating influence at least in determining the phototropic reactions of *Pilobolus*'. It is noted in the experiment upon which this conclusion is based that the blue rays are balanced against the sum total of all the rays included in white light. From the relatively greater influence which the preceding pages of this paper have shown the frequency of light to exert upon *Pilobolus*, as compared to that of intensity, this conclusion from unmeasured quantities of light could be anticipated.

In a later paper, however, Miss Jolivette (1914, p. 119), using different kinds of light sources, states, '*Pilobolus* fires its sporangia in larger numbers towards the lights in which the blue rays are greatest. In other words, it is more responsive to actinic rays. The intensities in the different wave-lengths are not measurable.' She further says that the energy given off by the source of light apparently does not compare in effect with the distribution of the same in different portions of the spectrum. In the experiments using a 16-candle-power tungsten and a 32-candle-power carbon filament lamp the large majority of the sporangia went to the tungsten light, although its total energy was but half that of the carbon. From this she concludes that the differences in distribution in the spectrum outweigh in effect the differences in the total energy of the two sources, a qualitative conclusion which the present paper through quantitative methods has shown to be valid.

The writer will not endeavour to explain the physiological significance of either the Fechner or the Tröndle formula in relation to the reaction of *Pilobolus* to different quantities of light energy. That there is a physiological principle within the living protoplasm expressed in these formulæ

is beyond doubt. Furthermore, the present experiments show conclusively the existence of a definite relation of frequency to this change in the living protoplasm which produces response.

That the square root of the frequencies appears as a function of the reaction as calculated in the present paper is a striking fact in view of Nernst's results on the relation of frequency to response in electrical stimulation, where he found the response to vary inversely to the square root of the frequency (cf. Nernst, 1899; Verworn, 1913). Present observation would indicate that the light stimulus follows directly in the line of electrical stimulation.

The writer desires to make further investigation into the relations of response in these two fields of research.

#### SUMMARY.

1. *Pilobolus* responds to the light of all the regions of the visible spectrum.
2. The presentation time decreases gradually from red to violet. There is no indication of intermediate maxima or minima.
3. The presentation time does not vary in direct ratio with the measured value of the energy of the light in the different regions of the spectrum.
4. The presentation time varies in inverse ratio to the square roots of the wave frequency.
5. The product of the square root of the frequency times the presentation time, decreases with the decrease in the energy value of the spectral regions, and is an approximate constant for a given light-source.
6. The spectral energy in its relation to the presentation time may be expressed approximately in the Weber-Fechner formula, if the wave-frequencies be made a function of the constant.
7. The relation of the spectral energy to the presentation time may also be approximately expressed in the Tröndle formula, the wave-frequencies being made a function of the constant.

The writer wishes to express her appreciation and gratitude to Professor Charles F. Hottes for inspiration and encouragement during the development of this problem, which was undertaken at his suggestion. She also wishes to thank Professor Jacob Kunz and Mr. Karrer, of the Physics Department, for their interest and assistance in the control of the delicate instruments used in the measurements of light.

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# The Structure of the Cytoplasm in the Cells of *Alicularia scalaris*, Cord.

BV

M. F. RIVETT, B.Sc.

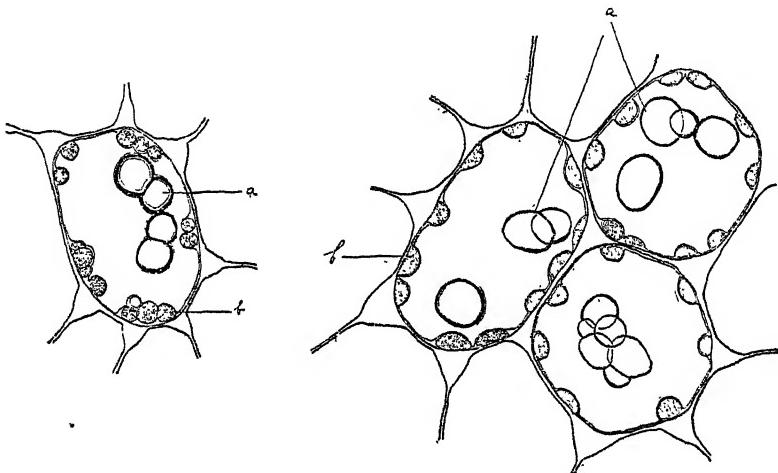
With Plate VI and three Figures in the Text.

THE tissues of the foliose liverwort *Alicularia scalaris* show, in common with many of the Hepaticae, the development of oil-bodies ; they are more conspicuous in this particular genus by virtue of their extremely large dimensions and high refractive index. The oil-bodies of the Hepaticae have been described by systematists as an aid to the identification of species ; they have been investigated in greater detail by Pfeffer (1), Wakker (2), Zimmermann (3), Raciborski (4), von Küster (5), Garjeanne (6), and the results of these investigations have been shortly summarized in biochemical works such as those of Czapek (7) and Molisch. Since, however, there still remain some doubts and contradictions in these results, particularly in connexion with the origin of the oil-bodies and their relation to the cytoplasm, this further research into the subject was undertaken.

The appearance of the living cells in the fresh mature leaves of *Alicularia scalaris* has frequently been described ; their shape is hexagonal, with rounded angles ; the walls slightly thickened ; their size is between  $25\mu$  and  $30\mu$  ; the chlorophyll granules are large and dispersed regularly and closely round the cell-walls ; the large oil-bodies, two, three, or four in number (but occasionally more numerous), are suspended in the midst of the cell and occupy the greater part of the cavity ; the nucleus is not visible in the living cell (see Text-fig. 1).

In a young living leaf, such as the second or third from the growing point, mounted and examined in water, the cells are seen to be very much smaller, about  $7\mu$  or  $8\mu$  in diameter. In the extreme apical and basal cells there is no trace of the oil-bodies, though there are minute refractive granules which appear at first to be of similar material (see Pl. VI, Fig. 1). They are continually in motion, revolving and rotating in the substance of the protoplasm. These, however, are found to persist unchanged throughout the development of the cell ; they never have any close association with the developing oil-bodies, and contrast with them in their behaviour towards fixing and staining reagents.

In a slightly older cell of the same leaf, the first traces of the oil-bodies (visible in living material) are to be found ; they appear as small refractive drops, similar to the minute granules of the smaller cells, but larger in size ; they may be scattered throughout the protoplasm or be limited to a small patch in the cell ; where they occur they give the cytoplasm an emulsified appearance similar to that of the oil-bodies in *Radula complanata*. They lie within the protoplasm and do not push through into the developing central vacuole. These oil-drops are in many cells continually in motion, rotating and revolving with the granules first seen in the younger cells, though less rapidly and in a smaller orbit. Owing to the high refractive index of the oil, the actual limits of their margins are obscured and they appear spherical with a dark edge. From their first appearance, their



TEXT-FIG. 1. Cells taken from mature leaf mounted entire in water and viewed from the surface. *a* = oil-bodies; *b* = chlorophyll-granules.

method of movement, they appear simply as drops secreted by the protoplasm within its own substance. There is no specialized external covering visible, for the protoplasmic surface from which they were secreted is blurred by the refractive glimmer of the oil (Pl. VI, Figs. 3 and 4).

In a cell which is a little more advanced than the previous one, the oil-drops have grown larger and their motion has diminished ; a few still appear to rotate slowly, but the revolving movements about the cell have ceased and each drop remains in its own place. In some cells at this stage there are distinctly two patches of oil-drops, foreshadowing the formation of two oil-bodies ; but this is not regularly so, and the whole surface of the cell may appear as one emulsified patch. At this stage the central vacuole of the cell is well developed and the protoplasm is retreating towards the cell-wall.

In a cell of an older leaf the central vacuole is nearly fully formed and the pressure exerted on the protoplasm also affects the collection of oil-drops.

Thus we find that the numerous small drops have coalesced into fewer larger bubbles. These are still within the substance of the protoplasm, separated from the cell-sap by a protoplasmic membrane. This is not visible owing to the refractive glimmer of the oil.

In the mature cells, i. e. those of the fully grown leaves, the large oil-bodies are visible. They protrude from some point on the cell-wall into the central vacuole; it is obvious from their position that they are bounded by a surrounding sac of protoplasm which is continuous with the primordial utricle lining the cell-wall. Thus the two secretions of the protoplasm—the cell-sap and the oil—are separated from one another by a partition of the mother substance. In these mature cells there are still to be seen the moving refractive granules found in the youngest living cells.

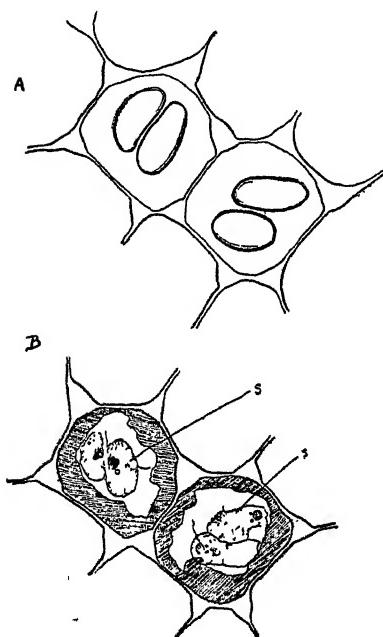
From these simple observations on the living material but few deductions can be made. There is certainly a suggestion that the oil originates as a general protoplasmic secretion, which appears as a series of bubbles; there is nothing to indicate that any special body (or elaioplast) is associated with each bubble or that the bubbles are localized to any particular part of the protoplasm; in fact, appearances are rather against such a view, because the numbers and disposition of the first-formed bubbles differ greatly in different cells. On these grounds both the small drops and later the coalesced larger drops can be termed oil-vacuoles, since they are nothing but spaces in the protoplasm filled with the oily liquid. Their formation is quite similar to that of the central vacuole, for the secretion of the drops one by one and the maintenance of their identity for some time are simply due to the physical properties of the liquid oil and its non-miscibility with the more fluid cell-sap; there is nothing to prove that the cell-sap is not secreted drop by drop within the protoplasm and afterwards collected into the large drop which fills the central vacuole. Whether the lining layer of protoplasm in contact with the oil is in any way changed by the contact or by its own act of secretion, so as to form a definite membrane, different in physical and chemical properties from the rest of the cytoplasm, cannot be determined merely by simple observation.

The nature of the oil in the oil-bodies is agreed upon by all investigators as a mixture of an ethereal oil with a small quantity of fatty oil and a proteid substance. This is proved by the action of alcohol, which will, in the cold state and diluted with water, dissolve the greater part of the oil with rapidity. If a leaf is treated with caustic potash, or with a mixture of caustic potash and ammonia, or with a solution of sodium carbonate, the oil-bodies do not lose their refractive properties; i. e. they do not saponify and therefore do not consist mainly of fatty oils. After treatment with alcohol it is always found that there is a small insoluble residuum consisting of an emulsion of small drops with a high refractive index: this seems to be partly a fatty substance and partly proteid. From the homogeneous appearance of the

fresh oil-bodies, it would seem probable that these substances form a solution with the ethereal oil.

The behaviour of the oil-bodies on treatment with alcohol is interesting and peculiar; if a living leaf is mounted in water, the behaviour can be observed by irrigating with 70 per cent. alcohol under the cover-slip. It has already been described by Garjeanne, Pfeffer, and von Küster. Pfeffer considered that the obvious membrane which is formed around the limits of the oil-body as the oil disappears is a wall or skin different from the surrounding cytoplasm. The wall can be stained with methylene blue and

with iodine and looks a very distinct and conspicuous structure (see Text-fig. 2). If leaves are mounted in 30 per cent. alcohol, the process of solution of the oil can be observed, as in this strength it takes place slowly. The oil-drop may divide into two, three, or numerous drops, and each of these becomes smaller; or the whole oil-drop may rapidly become spherical and gradually contract. The 'membrane' appears around the original limits of the oil-body. If, after the process of solution has gone on for a few moments, the slide is flooded with water, the membrane still appears conspicuous, and the partially dissolved oil-drops remain without further change. The process of solution is never complete in 30 per cent. alcohol.



TEXT-FIG. 2. A. Two cells with oil-bodies in surface view. B. The same irrigated with alcohol. *s* = 'sac' of oil-drop.

Von Küster (5), by plasmolysis experiments, satisfied himself that the membrane was an artifact, and by dissolving out the oil in two successive

stages, first with weak alcohol and then with strong, showed that two membranes could be formed, one inside the other. Garjeanne (6) showed that the membrane can also be made visible by pressure on the cover-slip, and as a deduction from this in conjunction with von Küster's experiments maintained that the so-called 'wall' (of Pfeffer) is a precipitation membrane formed by the action of the oil on the cell-sap; thus the 'wall' is discovered whenever the protoplasm separating the oil and the cell-sap is destroyed.

It seems to me that unnecessary stress is laid on the point whether the membrane is a differentiated wall or merely a protoplasmic binding. All are agreed that there is a protoplasmic covering round the oil; it is invisible

when the oil-body is intact owing to the refractive glimmer of the oil; when this is removed, the membrane shows up, probably with the addition of a slight precipitate; that its surface would be changed by contact with the oil is extremely probable; hence it absorbs a stain with more readiness than the remaining protoplasm. The formation of a second membrane on a second treatment with alcohol does not always take place, and is not nearly so conspicuous as the first, having more the appearance of a deposit of oil in a cavity.

I think we may safely conclude that the particular kind of membrane which is found around the oil-body does not present any argument against the idea that the bodies are vacuoles; the membrane is either the limit of the undifferentiated cytoplasm, or the surface of the cytoplasm slightly changed by contact with the oil. It is not an organ of sufficiently definite morphological and physiological characters to warrant the use of the word 'plastid' in connexion with the oil-bodies.

This simple method of secretion into vacuoles agrees with the fact that the oil is a catabolic waste product. The production is unaffected by any cultural conditions; i. e. by variations in light, temperature, and nutritive materials. It is therefore quite easy to imagine the whole thing as a simple chemical process—the oil-bubbles being given off from the reagents, just as a bubble of gas emerges when an acid touches a metal.

It may be objected that the regularity of form of the mature oil-bodies and the little variation of number in the cells produce a distinctness and individuality which argues against their description as spaces or vacuoles. This objection will certainly have weight with those who have observed the mature bodies only, but it cannot be maintained by those who have observed the emulsion of bubbles, out of which the oil-body arises. The constancy of form of the oil-vacuole is due to the equilibrium between the density of the oil and the density of the surrounding protoplasm. Under certain conditions a bubble can only take up one shape, and it is this which is seen in the constant form of the oil-bodies.

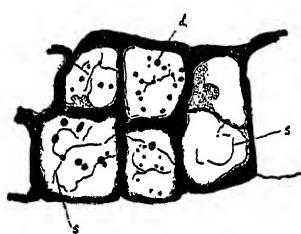
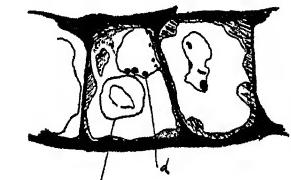
Garjeanne (6) describes the methods which led him to conclude that the oil is secreted in a vacuole; by means of picric acid, which colours the cytoplasm yellow and leaves the spaces uncoloured, he discovered minute vacuoles into which the oil is poured. I have repeated his methods with similar results, but do not place much reliance upon them, since no other fixatives produce the same appearance, and it is obvious that picric acid so rapidly causes change and disorganization in the cytoplasm, that it may easily lead to false conclusions.

The results of irrigating an entire leaf with 2 per cent. osmic acid while under the microscope, and also the investigation of entire leaves fixed in osmic for some hours, confirm the view that the oil is secreted in vacuoles. Both the first-formed drops and the mature oil-bodies stain homogeneously brown and stand out clearly from the surrounding protoplasm. If a series

of longitudinal sections through the growing point, cut in wax, from material fixed with osmic acid for twenty-four hours, and taken up through alcohol and xylol, are examined on the slide, mounted up in Canada balsam without further staining, then the only cytoplasmic bodies which show up clearly are the refractive granules. They show up as minute black spheres, which are more numerous in the cells of the growing point than in those of the older leaves, though they are still present in the latter. They are arranged in the peripheral layer of the cytoplasm and are also associated with the empty sacs which mark the limits of the oil-bodies. Thus they must be of different material both from the ethereal and fatty oils which disappear in the processes of dehydrating and embedding, even though partially fixed with

osmic acid. There is no indication in such an unstained slide of the vacuolar structure of the protoplasm which would indicate the formation of the oil-bodies.

After bleaching with hydrogen peroxide and staining with the triple stain (safranin, gentian violet, and orange G) certain other points emerge. The refractive granules stain brightly with safranin (thus resembling the nucleolus), but they are now much more conspicuous in the older cells than in those nearer the growing point (see Text-fig. 3). They are associated with the empty oil-sacs of the mature cells and are also arranged around the cell-walls. In the growing point they are very much smaller and are frequently masked by the orange colour, which stains the gel material of the cytoplasm. The cells of the



TEXT-FIG. 3. Mature cells in longitudinal section from base of full-grown leaf. *s* = sacs; *d* = granules stained with safranin.

growing point are filled with stained contents, which are mainly aggregated in the nucleus, whilst around it is a more or less homogeneous or exceedingly fine granular mass. There is no indication of a spongy network or of a vacuolar structure; the material of the cytoplasm consists of masses of grains so exceeding fine as almost to make up a homogeneous substance. This may be because both the grains and the gel in which they are embedded stain in the same way (see Pl. VI, Fig. 5 *a*, 1, 2, and 3).

The upper cells of the first two leaves show more differentiated contents; the nucleus still appears large in comparison with the total bulk of the cell and usually occupies a central position. There is a slight indication of vacuolar structure around the nucleus, but in the cells of the third and fourth leaves this is more evident (Pl. VI, Fig. 5 *b*, 4). In between the central nucleus and the peripheral layer of chlorophyll granules there is a vacuolar structure resembling the familiar 'spongy network' of the proto-

plasm. The spaces of the net are clear, and are separated by threads and wefts of stained material associated with more deeply stained granules. In the cells of fully-grown leaves this structure has disappeared, the protoplasm is restricted to a thin layer, lining the cell-wall, and from this the films or sacs of the oil-bodies stretch into the vacuole. These sacs are distorted and contracted owing to the changes of tension undergone during the embedding process. Around their edges are drops and granules stained brightly with safranin; some of these are undoubtedly the refractive granules of the cytoplasm, others have more the appearance of drops of glistening liquid which have taken up the stain. The appearance of these bodies is very constant in any preparation with the triple stain (see Text-fig. 3).

In a series of sections treated with iron-haematoxylon and iron-alum the characters of the cytoplasm become even more distinct. In the apical cell and those adjacent to it, the nuclei with their nucleoli are large blue-black bodies; the surrounding cytoplasm consists of a colourless (i. e. not stained with the haematoxylon) ground in which are numerous small, disconnected bodies, some round and spherical, others thread-like, others irregular, but all are stained deeply grey or black with the haematoxylon. They seem to represent the chondriosomes, or chondriome-structure of the cytoplasm, which has been described in various plant tissues by Schmidt (8), Guilliermond (9), Lewitzky (10), and others. In the upper cells of the second pair of leaves folded around the growing point, the structure is different; around the bulky nucleus is a network formation; the strands of the net are stained deeply grey and the meshes are lighter or colourless; at the crossings of the strands and occasionally around the cell-walls are minute round bodies stained still more deeply. In the next pair of leaves this structure still remains, but in the fourth and succeeding pairs the development of the large central vacuole leaves the cytoplasm only adhering to the cell-walls. In some few cells the nucleus is visible, and in all there is a large development of chloroplasts. Occasionally the 'sacs' of the oil-bodies are stained faintly grey, but they do not readily absorb the iron-haematoxylon. Associated with them and occurring also around the cell-walls are round bodies, which are faintly stained and not very conspicuous; these are the bright, refractive bodies of the living protoplasm which stained most clearly with safranin (Pl. VI, Fig. 6 and 7).

To sum up shortly the cytoplasmic structure of the leaf-cells:

- (i) the actively dividing cells of the growing point and young leaf bases, where the protoplasm fills the whole cell, show a chondriome structure;
- (ii) the maturing cells have a vacuolar protoplasm forming a 'spongy network';
- (iii) in the fully-grown cells, the lining layer is so reduced that it serves merely as a covering to its own products.

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There is no evidence from my observations to show that the chondriosomes are either transformed directly into plastids by a secretion within their own substance, as has been observed in other plant cells, or that they are the instigators of secretory action on the part of the protoplasm. Nor do my observations suggest that the refractive moving bodies of the living protoplasm are themselves chondriosomes, for their appearance in the stained mature cells is quite different from that of the chondriome in the actively dividing cells. This investigation merely supports the view that the cytoplasm of the actively dividing cells has a chondriome structure and is not a spongy network, though the vacuolar appearance of the older cells might quite well be given that name.

Finally, I wish to express my thanks to Professor J. B. Farmer, F.R.S., both for the suggestion of this research and for the assistance which he has rendered.

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EXPLANATION OF PLATE VI.

Illustrating Miss Rivett's paper on the Structure of the Cytoplasm in the Cells of *Alicularia scalaris*, Cord.

Figs. 1, 2, 3, 4. Very young leaves mounted in water and viewed from the surface, showing early formation of oil-drops.

1. Apex of second leaf: three cells without oil-drops, one cell with oil-drops.
2. Margin of third leaf near base.
3. Margin of third leaf nearer apex.
4. Apex of third leaf.

Figs. 5, 6, 7. Cells from longitudinal sections through the growing point.

- 5a. Apical cell of first and third, 5 b, of fourth leaf stained with triple stain.
6. Apical cells showing chondriome structure, stained with iron-haematoxylin.
7. Cells from fourth leaf showing vacuoles and young chloroplasts.

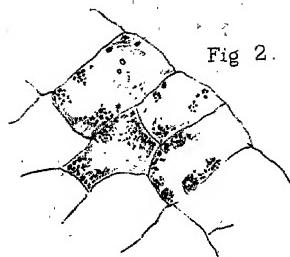


Fig. 2.

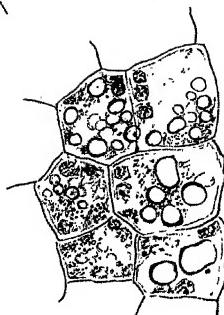


Fig. 3.

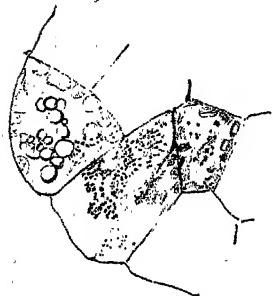


Fig. 1.

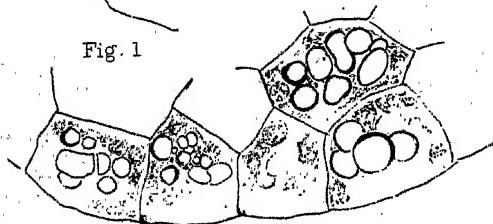


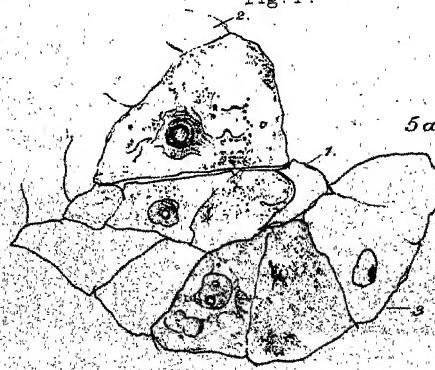
Fig. 4.



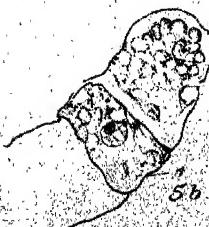
Fig. 6.



Fig. 7.



5a.



5b.

Fig. 5.

Smith, N.M. et. al.



# Fragments of the Flower Biology of Westralian Plants.

BY

OSWALD H. SARGENT.

DURING the past twelve years I have paid special attention to the floral biology of just a few of the multitude of species constituting the flora of Western Australia. It is proposed in the present paper to state briefly the conclusions reached, and incidentally record a few isolated scraps of information that otherwise might not see the light. It seems most convenient to group the facts under the heads of pollination agents, commencing with

## ORNITHOPHILOUS FLOWERS.

These are placed first because I have come to look upon birds as the most important agents of pollination in this country. Many flowers which at first struck me as entomophilous have on further investigation appeared to be specially adapted for birds. Observations of the habits of honey-eating birds have led me to think it possible (indeed even probable) that adaptation of the flower to their requirements is the price of the plant species' life. Not so much because of the advantages of ornithophily ; but rather because of the disadvantages of any other course. Honey-eaters are aggressive, and if a flower's store of nectar is not readily available they rip open the blossom to secure it. Nectar need not be particularly copious to attract birds ; and I have seen the corollas of several cultivated plants badly damaged by them. It is of interest that species of *Erica* and *Arbutus* suffer severely. My observations lead me to conclude that in order to escape the attentions of birds a flower must be nectarless or nearly so, or so situate on the plant as to be practically inaccessible to birds. I do not wish to argue that ornithophily is not directly advantageous ; but simply to state that I have not seen unequivocal evidence of its positive advantages, though I have seen much of the negative. To state the matter in another way, the impression left is : given a species with varying forms of flower, one extreme form being best adapted to insects and another to birds, the latter will survive, because the birds will so injure the former that few will secure pollination. The one point that has struck me as possibly of direct advantage is the relative simplicity of structure adequate to secure pollination when birds are the

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pollinators. Calyx and corolla may be absent from the open flower, or, if present, reduced to mere scales. In such cases the stamens are numerous; but when simplicity is attained by reduction of the perigone to scarcely more than a funnel-shaped tube the stamens are few. In most cases the style is slender, often quite filamentous (but usually very rigid), and the stigma a mere point. This reduction of parts is doubtless an advantage in an arid country such as this; but still my present impression is that the aggressiveness of birds has had more to do with the survival of so many ornithophilous plants, or is responsible for the prevalence of ornithophily, in this Southern Land. What has struck me more than anything else as the outstanding characteristic of bird-pollinated blossoms is the rigidity of the parts. The anthers are often almost woody, and, disregarding versatility, are usually only moved from their normal position with some difficulty because of the rigidity of the stamens. The style, if fleshy, is sufficiently thick to be rigid, and if slender is sufficiently woody to be springy or stiff.

In dealing more particularly with instances of bird pollination that have come under my notice it will be most convenient to classify the facts according to types of floral structure, proceeding from the simple to the complex. There is, right at the outset, a difficulty in judging; but I think the brilliant orange flower of *Nuytsia floribunda*, R. Br., is the least specialized on my list. It is practically tubeless as the petals with the attached stamens spread rather widely from their bases, so that the nectar is well exposed. The simple erect subulate style is crowned by a minute apical stigma, which appears to be ripe at anthesis simultaneously with the anthers. The only trace of dichogamy I have noticed is that sometimes a few of the anthers do not dehisce on the day the flower opens. I have repeatedly watched hive-bees visiting the flowers; but though they often brush against the anthers I have never seen one touch the stigma. On just a few occasions I have seen a bird (honey-eater) visit the flowers. My observations and the structure of the flower incline me strongly to the opinion that birds are the 'official' pollinators, if that convenient and expressive term may be permitted me. My experience has made me desire a word to describe that visitor which seems specially connected with a flower. Others may be efficient pollinators; but one appears to be *the one* for whom the blossom is 'intended'. The parts of the *Nuytsia* blossom are rather rigid, which points to a vigorous pollinator. The flowers are borne in immense trusses; and, when a bird alights on a truss to sip the proffered nectar, it is highly probable that wholesale pollination takes place.

The flowers of *Loranthus linophyllus*, Fenzl, are slightly more specialized. Petal bases and epipetalous stamens are suberect, a short but definite tube is formed by the connivent bases of the petals, and the blossom is definitely proterandrous. I have many times seen birds (*Zosterops gouldi*, B'parte, and other honey-eaters) sipping nectar from the blossoms of this

plant, which is plentiful about York as a parasite upon *Acacia acuminata*, Benth. The flower is over-large for any insect—it is about an inch in length—but in dipping into it for nectar a bird can scarcely fail to receive pollen on some part of its head, or to leave some on a stigma. The inflorescence is a pendulous tangled tassel of petals, stamens, and styles. The flower parts are rigid; the style, though filamentous, is remarkably tough and springy, and seems well adapted for scraping pollen from feathers. It is worthy of note that this species is doubly ornithophilous: its seeds are distributed by birds, which devour the sticky-fleshed berries, and wipe the naked seeds off their bills on to convenient twigs. The plant flowers in December, and fruits ripen about March.

Closely similar in structure is the flower of *Xanthorrhoea Preissii*, Endl., the 'Black Boy' or 'Grass Tree'. The erect perigone scarcely extends beyond the general surface of the spike (the bract tips); the stamens spread, but not widely; and the style does not attain its full length till some days after anthesis. A glistening drop of nectar occupies the centre of each flower, the style leaning to one side. As I have frequently seen small birds clinging to the gigantic spike, and dipping their beaks into the flowers, I think they often, if not usually, act as pollinators, though the flowers are freely visited by many insects.

The delicate blossoms of *Acacia celastrifolia*, Benth., do not at first sight appear to be at all adapted for bird pollination, yet my observations have led me to regard birds as the official pollinators. The only visible part of the open blossom is a multitude of flimsy stamens so disposed as to form a fluffy ball. The flowers are borne in subsessile pairs on racemes about three inches long inserted in the axils of the phyllodes. The source of attraction is a gland on the upper edge of the phyllode, which secretes nectar at flowering time, and only then. *Zosterops gouldi* and *Glyciphila* spp. are frequent visitors, and in sipping nectar seldom, if ever, fail to brush against several flowers. Hive-bees visit the glands in swarms, but are too small to brush against the anthers. The raceme does not stand directly over the phyllode edge, but in a plane about  $30^{\circ}$  distant; so that, while birds are given thereby freer access to the nectar-gland, the visits of nectar-seeking insects are sacrificed. Bees gather pollen at times, and then probably act as pollinators. A more complete account of my observations will be found in the 'Journal of W. A. Natural History Society', No. vi.

Species of *Eucalyptus* come next on my list. Sepals and petals are discarded at anthesis in the form of a calyptra in this genus, and the open blossom is in some respects very simple in structure. A ring of numerous thread-like stamens surrounds the top of the ovary, which is surmounted by a slender terete style, whose apex only is stigmatic. A few figures recently obtained from a flower of *Eucalyptus macrocarpa*, Hook., will be much more effective than a long description in revealing the character of the flowers.

I estimated the number of stamens at fourteen hundred. Their bases occupied a band round the ovary top  $2\frac{1}{2}$  mm. wide, while their anther-bearing tops spread to a width of about 25 mm. The middle circumference of the stamen ring taken over the anthers was 140 mm. The stigma was less than a millimetre in diameter! The area of the pollen-bearing surface was therefore over three thousand times the area of the surface adapted for receiving pollen. In this estimate I have included the spaces between the anthers; but even if that inclusion be disallowed the ratio would still be enormously disproportionate. What could be more eloquent of the low degree of specialization of this flower? I have no positive knowledge that birds are the chosen agents of pollination in this particular species, but I feel no doubt whatever that such is the case. While the above is perhaps an extreme case in all species of *Eucalyptus* the area of the pollen-bearing surface is enormously out of proportion to the area of the stigma, which is always minute. This points, I think, to a pollinator of comparatively large size. The top of the ovary secretes nectar, often very copiously, so that it falls in drops from the flowers. Birds seeking this nectar would certainly be liberally dusted with pollen, and could scarcely fail to bring some of their pollen-bearing feathers into contact with the stigma. I have many times seen small parakeets busy on the flowers of *E. redunca*, Schau., and *E. accedens*, W. V. Fitz. Once I observed *Zosterops gouldi* sipping nectar from the flowers of *E. loxophleba*, Benth. The only species I have observed at all closely is *E. calophylla*, R. Br. Its flowers are freely visited by insects seeking the very copious nectar or pollen. Various honey-eating birds are also frequent visitors. The latter appear to be efficient pollinators; but the insects seem useless: I have never seen one brush against a stigma, though I have watched long and carefully. I have found Eucalypts troublesome to observe, and I regard my present knowledge of their flower biology as very incomplete. Yet what I have so far seen has impressed upon me the belief that birds are the chief pollinators of the genus. The small size of some of the flowers at first led me to regard them as entomophilous, but I am now satisfied that birds could and would take nectar from the smallest, and in so doing would almost certainly effect pollination.

The flowers of *Beaufortia sparsa*, R. Br., show some degree of special structural adaptation to bird pollinators. Sepals and petals are much reduced: bud and ovary protection seem their only functions. The stamens, bright scarlet in colour, are collected into five slender bundles about an inch in length, only their tips being free; the anthers are minute; and the slender style, somewhat longer than the stamens, is tipped by an almost microscopic stigma. I often saw small honey-eating birds busily sipping nectar from the flowers, during visits to Albany in 1909 and 1910. The anthers freely rubbed against cheeks, foreheads, and throats; and I concluded that the birds were very efficient pollinators. This type of floral

structure—the reduction of the perigone and marked development of the androeceum—reaches its culminating point in the flower of *Calothamnus sanguineus*, Labill. The two upper stamen bundles are united into a broad half-tube, gradually becoming an almost flat band towards its apex, where it breaks into a fringe of short filaments. This structure is inclined towards the ground, so that the anthers are in a plane below that of the floral axis, though the bundles are inserted above it. The anthers are thus caused to press against the head of any bird taking nectar. The remaining two bundles are reduced each to a single filament, shorter and with smaller anthers than the others. At first sight these reduced bundles seem useless; but I have observed that their anthers brush the bird's cheek. Now the stigma usually rubs the bird's head; but it is easily displaced and it may sometimes rub the cheek. If so, the value of the reduced lateral bundles is obvious. My observations are insufficient to show whether this more highly specialized plant is more successful in securing pollination than *Beaufortia sparsa*. Both species fruit very freely.

The bright red tubular flowers of *Astroloma divaricatum*, Sond., are less than half an inch long, and the tube is only about one-eighth of an inch wide. The floral mechanism closely resembles that of *Erica* spp. I at first thought the flower entomophilous; but though I have kept an eye on the plants for many years I have not observed insect visits. On the other hand, I have often seen small birds on the shrubs at flowering time, and on one occasion my brothers saw a bird 'suck honey from a flower'. If this species is indeed ornithophilous, other species of *Astroloma* are probably so also. My observations of bird visits to *Arbutus* and *Erica* flowers are of interest in this connexion. I have not seen *Astroloma* flowers damaged by birds. Quite recently I saw a small bird thrusting its beak into the flowers of *Habrothamnus* sp. in a Perth garden. These flowers are comparable in size and shape with *Astroloma* blossoms, and were not damaged. I could not make out whether pollination took place.

The plant's close relationship with *Anigozanthus*, and the size of its tubular red blossoms, lead me to think that *Blancoa canescens*, Lindl., is probably pollinated by birds. The flowers of most species of *Anigozanthus* are tubes split open on their lower sides. In the species best known to me, *A. humilis*, Lindl. (flower orange and red), *A. bicolor*, Endl., and *A. Manglesii*, D. Don (flowers rich green with scarlet base, in both species), the perigone might well be likened to a ribbon fastened round the ovary at one end and having the other free and practically flat. Just below the flat end six linear anthers are set in an orderly row on very short filaments. The style is very slender, longer than the perigone, with somewhat swollen apex and apical stigma. I have never had the good fortune to see the flowers visited. My brothers, however, have repeatedly

seen the flowers of *A. bicolor* visited by small birds (*Glyciphila*?), and some years ago friends in South Perth told me they had seen small birds assiduously taking nectar from the flowers of *A. Manglesii*. I have spoken of the flower of *Calothamnus sanguineus* as the culminant of one type of floral structure. It is worthy of note that an almost identical arrangement (mechanically and in appearance) is reached by the flowers of *Anigozanthus*, which I think may be regarded as the culminant of the tubular perigone type of structure. Further development would require an alteration of plan.

Four papilionaceous plants I believe to be ornithophilous : *Templetonia retusa*, R. Br. (red flowers with yellow eye), *Crotalaria Cunninghamii*, R. Br. (green flowers), *Clanthus Dampieri*, A. Cunn. (red flowers with black eye), and *Kennedyia nigricans*, Lindl. (black flowers with yellow eye). I have only actually seen birds at work on the flowers of the first named; but the size and rigidity of all seems too great for insects. Structure and mechanism call for no special comment.

I come now to my last type, I think the most specialized of all. In this the style apex before the maturity of the stigma acts as pollen presenter. The perigone does little more than protect the essential organs in the bud. In some of the less specialized cases it assists perhaps in attracting pollinators. In many cases its base forms a useful nectar container. The anthers discharge their loads of pollen on to the style top before the flower opens, and are withered shells in the open flower, which consists essentially of a slender filament or rod bearing first pollen, and, later, a microscopic stigma. Surely that is simplicity *in excelsis*, and speaks eloquently of triumph over dry and trying conditions, because the simplicity is clearly not primitive but developed. The only plants I know in this class belong to the Natural Order *Proteaceae*. The multitude of individual forms is bewildering. Almost every variation occurs from the simple solitary flower through all degrees of aggregation to the compact spike or even a capitulum. I have notes concerning but few forms.

*Adenanthes cuneata*, Labill., has simple flowers solitary in the axils. They are dull red and seem small for the attentions of birds; yet I often saw birds—*Zosterops gouldi* chiefly—dipping their beaks into the flowers when in Albany a few years ago. The protruding style end rubbed the bird's head, and pollination seemed inevitable. This plant is interesting in that while the flowers are small and inconspicuous, the subtending leaves are of a crimson hue, and the branch tips bearing them, especially when seen against the light, resemble racemes of flowers. The behaviour of the birds, however, did not suggest that they were attracted by the bright colour. The flowers of species of *Grevillea* are closely similar in structure to those of *Adenanthes*, but they are aggregated in loose racemes, and perhaps render some mutual aid in the way of attraction.

The coral red blossoms of *G. bipinnatifida*, R. Br., and the brilliant scarlet ones of *G. Wilsonii*, A. Cunn., are almost certainly bird-pollinated: their styles project too far from the perigone for insect pollinators to be efficient. The inflorescence of *Hakaea myrtooides*, Meissn., is highly specialized. I have, indeed, felt some inclination to set it down as higher than the forms next to be considered; but, as each branch bears many inflorescences simulating a raceme, I have decided it is inferior to those forms whose branches bear one terminal inflorescence. It is a diffuse subshrub. The last few inches of each branch bears in every axil an umbel of from three to six tiny blossoms of ruddy purple hue. The perigone is but one millimetre across and four millimetres high, yet the style is twelve millimetres in length. The posterior side of each floweret, which faces the umbellar axis, is split to the base where the tiny disc (about 0.12 c.mm.) protrudes. The secretion of nectar is out of all proportion to the disc's size: I have found drops measuring at least 60 c.mm. resting between five flowers. I do not think rain could have contributed: the drops were quite sweet, and drops of water carefully placed in the centres of umbels always quickly disappeared, finding their way down the stem. The nectar would certainly attract birds; and the flower seems ill adapted for insect pollinators. The bird which could sip a nectar-drop without brushing at least one of the erect (or slightly incurved) styles surrounding it would be skilful indeed.

*Grevillea eriostachya*, Lindl., will serve well as a connecting link with the most highly specialized inflorescences. Its yellowish flowers are borne in dense secund racemes terminating the branches. Though the flowers are packed very closely together, the stigmas all face one way—towards the apex of the stem—so probably each flower would need individual attention from a pollinator. A bushman recently remarked to me that though the flowers secrete nectar very freely—boys are fond of sucking the flower spikes and call the shrub 'honeysuckle'—bees very rarely visit them. Doubtless birds are the official pollinators.

The inflorescence of *Banksia* spp. is a large somewhat cone-like spike, singularly beautiful in appearance, and often brightly coloured. The flowers are so densely crowded together as to form almost a solid mass from which the hard tough styles project. I have many times seen small honey-eaters taking nectar from the flowers of *B. attenuata*, R. Br., *B. prionotes*, Lindl., and *B. Menziesii*, R. Br., and cannot doubt that they act as pollinators. The bird usually perches on the top of a spike and bends down to take nectar, probably brushing many styles which point upwards in the process.

The highest degree of specialization is found, I think, in those species of *Dryandra* whose inflorescences strikingly resemble, superficially, those of Compositae. Many times and often have I seen birds thrust their

beaks into the thistle-like heads of *D. floribunda*, R. Br., and *D. carduacea*, Lindl.

#### ENTOMOPHILOUS FLOWERS

now claim some attention. Though Lepidoptera are numerous here, I do not yet know for certain even one species of plant chiefly pollinated by either butterfly or moth: indeed I have observed extremely few visits of these insects to any flowers. On the rare occasions when I have seen a butterfly visit a flower it has seemed useless as a pollinator. The flower of *Stackhousia Brunonis*, Benth., seems to be specially adapted for moths. The corolla is a longish tube, and at night it exhales a most delightful, Narcissus-like perfume (Mr. C. R. P. Andrews first called my attention to this fact). The flower is yellow or tawny in colour, and does not seem particularly visible at night.

Indigenous Hymenoptera visit many flowers; but only in one case have I definitely connected one with a flower as official pollinator. The Orchid *Caladenia Barbarossae*, Reichb., is visited by a large black wasp, whose name I have not yet ascertained. The labellum of this Orchid is so arranged that when the wasp alights on the lamina it swings forwards towards the centre of the flower. This movement brings the wasp's back forcibly into contact with the stigma, whose secretion renders a circular patch of the wasp's back sticky. When the insect seeks to leave the flower, one edge of the sticky patch comes in contact with one or more of the disc-like pollinia peeping from the anther just above the stigma. Even momentary contact serves to attach the edge, and the whole disc is withdrawn from the anther as the wasp's egress continues. When withdrawal is complete the pollinium falls upon and exactly fits the sticky patch on the wasp's back. Obviously the next flower visited will receive upon its stigma a liberal supply of pollen. A full account of my observations will be found in 'Journal W. A. Nat. Hist. Soc.', No. iv (Nov. 1907). At that time I had not determined what is the attraction of the flower. Subsequent investigation has, I think, revealed it. The central labellar appendage, which the wasp seizes with its mandibles, seems to be a true gland, secreting nectar so slowly that crystallization takes place practically simultaneously. In one instance I found the crystals aggregated into tiny globular masses much resembling raspberries as I viewed them against the purple skin of the gland. The crystals appear to be sucrose. An amorphous sticky substance (glucose?) is also present. My investigations are at present very far from complete; but I have reason to believe that the peg-like calli of all species of *Caladenia* and allied genera secrete similarly. I have never yet found them gnawed by insects (excepting cases where a large portion of the labellum has been damaged—evidently not by a pollinator): I believe they are simply 'licked'. The genus *Drakaea* is highly interesting.

As it is closely allied to *Caladenia Barbarossae*, and is, I believe, pollinated by Hymenoptera, I will include a brief account of it here. Near York *D. ciliata*, Reichb., is plentiful. It grows round the edges of shelving granite outcrops where the soil is almost swampy during winter. It flowers in November, when the soil is very dry and warm, its fleshy scape dying from below upwards as flowering proceeds. *D. elastica*, Lindl., and its near ally *D. Glyptodon*, R. D. Fitz., occur sparingly on sand. Both flower in September. I have not observed the process of pollination in any of these species; but after careful and prolonged study of the flowers I have formed an idea of the mode of operation of the flower parts. The labellum, which in all three species is strongly suggestive of some weird insect perched upon the blossom, is thinly clothed with shaggy hairs, and is attached by a loose but tough hinge. In none of these species is there any trace of irritability in any part of the flower. The basal calli bear crystalline nectar. My conclusion is that when an insect settles on a labellum it is allowed a meal in peace; but when it seeks to leave, its legs being somewhat entangled by the shaggy hairs, it is temporarily attached to the labellum, and so is constrained to fly in an arc which quickly brings it into forcible contact with the stigma, where it soon manages to free itself; but not without removing pollen or pollinating the stigma. In the case of *D. ciliata* I actually induced a small hymenopt to perform part of the operation; but it was evidently not the proper pollinator.

The petaloid calyx of *Thomasia montana*, Steud., simulates the corolla of a *Solanum*. It is usually of a delicate bluish pink hue, though sometimes clear rose. The petals are tiny black scales and seem functionless. The stamen bases are connate, forming a shallow cup (this structure is peculiar to the species) on whose rim the horny anthers are almost sessile. These anthers stand close together and form a black cone from whose apex the slender style protrudes. The stamen cup contains thick nectar, to obtain which an insect must probe between anther tips and style. That done a further obstruction is met: the staminodes, which alternate with the fertile stamens, lean inwards and form a tangle round the style. Altogether a deal of poking is necessary before nectar is secured. This disturbs the anthers and dry, dusty pollen is shaken from their terminal pores. I have never seen an insect seek nectar, but I have seen small black indigenous bees gathering pollen from the flowers. They perched inverted (the flowers are pendulous) upon the anther cones, and agitating the anthers with their legs caused pollen to fall out. I concluded that they would effect pollination whilst so engaged. They are very active creatures, frequently flitting from shrub to shrub. True xenogamy is probably the rule when they act as pollinators. The flowers are, however, fertile with pollen from others of the same shrub. Some years ago I observed that a solitary shrub,

miles away from any congener, produced a few fruits from its wealth of blossom.

The rosy calyces of *Guichenotia Sarotes*, Benth., both in shape and colour are reminiscent of advanced inflorescence-buds of *Bougainvillea Sanderiana* of horticulture. The floral structure is essentially similar to that of *Thomasia montana*; but nectar and staminodes are both wanting. The anthers form a cone round the pistil, which is an object of most exquisite beauty. This beauty, however, is only revealed by a lens. The pale green ovary is sparsely clothed with glistening starry hairs, amongst which are dotted plump ovoid trichomes filled with purple sap. The style is so densely and delicately clothed with starry hairs that it appears to be surrounded by a halo. Description, I fear, fails utterly to convey anything like an adequate idea of the beauty. The purple trichomes are, I presume, either gnawed or sucked by visiting insects. Either action would doubtless cause sufficient agitation of the anthers to shake out pollen. I have not seen the flowers visited by any insect. The close relationship of the species with *Thomasia* induces me to make some mention of it here.

I now come to one of the choicest gems of the Westralian flora—*Leschenaultia biloba*, Lindl. It seems almost sacrilege to attempt a description, yet one, confessedly inadequate, must be offered. The flower suggests to me a bright blue ten-rayed satin star, crinkled and curved most elegantly. While celestial blue is the rule the shade of colour of the petals varies from almost pure white (*very rare*) to deepest ultramarine. Sometimes the bright blue blossom possesses a pure white 'eye', which at times occupies nearly one-third of the limb. The corolla is actually, though not obviously, two-lipped, and the 'eye' is always limited to the anterior lip of three petals. The anthers shed their pollen into the indusial pouch, then a gaping mouth, some time before anthesis. In the open flower the stamens are simply shrivelled shreds. The style is a thick column of tissue with a broadly expanded, slightly dorsiventrally flattened apex. The structure of the expanded part is remarkable. On its anterior edge there is a furry-looking whitish line, which indicates the closed mouth of the pollen-containing indusium. On the opposite side of the style top there is a broad slightly curved line of short fleshy hairs. The central space is occupied by a glistening, sticky, oval patch—the stigma. The edge of the indusium stands about one millimetre distant from the corolla tube. An insect entering the flower would first brush against the fringe of fleshy hairs and the stigma almost simultaneously; it would then brush against the edge of the indusium, and thus cause the pouch to open, and allow pollen to fall out, or at least expose it, so that some might be removed. I have spent much time in watching the plants; but only twice have I seen flowers visited. Once a small indigenous 'bee' entered a flower just as I have described; but the pouch was empty, so I did not see the complete operation. The

flower contains a fair-sized drop of nectar at the base of the corolla tube, yet hive-bees always pass the flowers and visit those of other plants, though they contain less nectar. I was puzzled by this strange behaviour till recently when I saw a hive-bee visit several flowers. Her head was blocked by the sturdy style, and I do not think her tongue reached the nectar-drop about 7 mm. distant. So far as I could see the pouch did not open. I discovered by accident not long ago that the flowers so blue by day appear brilliantly white in the moonlight. This appears to be due to the structure of the cuticle of the petals, making them excellent reflectors of light—so excellent, indeed, that the flowers appear brighter than many pure white blossoms. This fact suggests night-flying insects as pollinators. I have not yet been able to make any definite observations in this direction. I cannot think that Lepidoptera would prove efficient pollinators. The pollen is somewhat moist or waxy, and does not readily fall from the pouch, which, moreover, is broad-mouthed and seems intended for the back of a pollinator. This species is a very erratic fruiter. I have made some attempt to discover the reason. The plants commonly occur in groups or colonies. Careful digging reveals that the rhizome spreads widely underground and sends up aerial shoots at intervals. What seem to be several separate plants growing near together are often in reality simply branches of the same plant. I have found that the flowers are infertile with pollen from other flowers of the same plant. I have observed that when two distinct individuals grow in close proximity, both usually fruit freely; groups, on the contrary, seldom produce many fruits. This seems to show that the pollinator does not as a rule travel far between visits. In all other genera of Goodeniaceae known to me, viz. *Goodenia*, *Scaevola*, *Dampiera*, and *Brunonia*, the indusium surrounds the style apex, which pushes the pollen before it as it grows; and the stigma is not mature till the style tip has extended beyond the indusium and all pollen has gone.

Species of *Stylidium*, Sw., bear flowers in some respects more highly specialized than those of Goodeniaceae. Stamens and style are closely welded together into a highly irritable column, which in the 'set' position leans upon the much reduced fifth petal ('labellum') with its apex below the plane of the corolla limb. The labellum is morphologically anterior, but torsion of the corolla tube carries it to the side or back of the flower. The column is bowed forwards a little just as it emerges from the corolla, so that it almost occludes the entrance. Slight pressure (a mere touch is not enough) against this bowed part causes the column to move with great rapidity till it leans over on the opposite side of the flower. It soon returns to its former position, but does not regain irritability till after a short rest there. I have seen various hymenoptera visit the flowers and apparently obtain nectar without causing the column to act. Once I saw a largish 'bee' visit several flowers on a raceme in rapid succession, working from

below upwards. Not a single column flew over, though all were in the irritable condition! The back of this bee appeared to be pollen-dusted. I think the anthers or stigmas (according to the stage of flowering) usually strike the back of a visitor when the column reacts to gentle pressure. In some species the under-surface of the visitor may be struck. Anthers ripen first, and are pushed aside by the growing style tip just before the stigmas mature. The motion of the column is too rapid for the eye to follow.

A pollination mechanism recalling that I have described for species of Proteaceae is found in *Verticordia pennigera*, Endl., and *V. densiflora*, Lindl., and probably other species, but I have not specially observed them. The style is an erect column with an apical stigma. Just below the stigma is a ring of hairs; and on to this ring the anthers discharge their pollen-grains, floating in a sticky fluid just before the flower opens. The stamens are not functionless in the open blossom; they partially close the floral orifice and so make the nectar less readily accessible. The flowers are visited by hymenopters, which are, I believe, efficient pollinators.

The flowers of *Actinodium Cunninghamii*, Schau., which are borne in heads strongly suggestive of 'double' *Bellis perennis capitula*, follow a closely similar plan to that of *Verticordia*, and are, I believe, also pollinated by Hymenoptera.

The mechanical structure of the flowers of Compositae scarcely differs essentially from the above-named. I have seen the flowers of *Helichrysum Lawrencei*, F. Muell., visited by small hymenopters which seem to pollinate effectively.

*Marianthus lineatus*, F. Muell., depends chiefly for pollination upon a small dipteran, as I have shown at some length in 'Journal N. H. & S. Society of W. A.', vol. iii, No. 1. The plant blooms during the hottest months of the year—January and February. Its elegant blossoms, cream-coloured with purple longitudinal stripes, are borne in great profusion. In the bud, neglecting the pistil, the flower, even to a fairly late stage, is actinomorphic: sepals, petals, and stamens are regularly spaced in their respective whorls, and in each whorl the units are not perceptibly dissimilar in any respect. When the blossom opens, though their claws are still almost equally spaced round the sporophylls, the limbs of the lateral and anterior petals are bent back, so that the complete corolla forms a half-bell only at the back of the flower. The stamens now differ in length and are so arranged that their anthers form a compact little cushion facing the petals. About four days after the flower opens the small stigma will be found occupying the place of the anther cushion, the stamens having spread widely away from their first position. Nectar is not secreted; but the tiny teat-like trichomes, which thickly stud the petal bases, contain a sugary juice. My observations and experiments point to the conclusion

that the flies obtain the juice by squeezing or sucking it through the cell-walls without injuring them.

A genus of small terrestrial Orchids, *Pterostylis*, has received considerable attention from me. As I have given a fairly full account of my observations in 'Annals of Botany', vol. xxiii, p. 265, the briefest mention is sufficient here. T. F. Cheeseman in 'Trans. N. Z. Inst.', vol. v, was, I believe, the first to give an account of the mechanism of the flower. R. D. Fitzgerald also describes it in his great work, 'Australian Orchids'. My own observations and conclusions were, however, made and reached independently before having read the account given by either of those authors. Further observations and experiments have tended to confirm my conclusion that the flowers are pollinated by gnat-like Diptera.

*Cephalotus follicularis*, Labill., claims inclusion here on account of its intrinsic interest, though my knowledge of its flower biology is far from complete. The inflorescence is a narrow panicle of small starry white flowers. It is borne upon a remarkably long foot-stalk, which serves to elevate it well above the sedges and low shrubs closely surrounding the plant. The calyx is a shallow cup with limb divided into six triangular lobes. Each of the twelve stamens has a comparatively large gland, strongly suggestive of a miniature strawberry, at the back of the anther. When the flower opens the stamens are reclined, and their glands form two neat rows round the conical pistil, whose style tips are closely folded together. Soon a few (2, 3, or 4) of the outer row of stamens unbend and their anthers dehisce. The rest come into action on the following day. The stamens of the inner row behave similarly on the two succeeding days. Then the style tips spread out and the stigmas ripen. The anthers have usually lost all their 'moist' or waxy pollen before the styles diverge. The flowers exhale a faint perfume reminiscent of fine honey. There is no free nectar, but the inner surface of the calyx tube is studded with glistening papillae containing sugary sap. I have seen the flowers visited by hymenoptera and by Diptera. All the visitors seemed equally efficient as pollinators. The inflorescence is clearly visible at a distance of 30 yards, appearing as a pale grey smudge upon the surrounding verdure. I made a rough estimate of its relative visibility by setting up near an inflorescence a raceme of small yellow flowers (*Comesperma* sp.) and one of scarlet *Beaufortia*, both trimmed to the size of the *Cephalotus* panicle. The last named was quite as visible or conspicuous as the yellow flowers, but somewhat less so than the scarlet to my eye. The flowering season was not over when I left the locality; but I found twenty-three panicles that had completed their course. Thirty-eight per cent. (261) of their flowers (696) had produced follicles.

Only one anemophilous plant—*Opercularia vaginata*, Labill. (N.O. Rubiaceae)—has received any attention from me. The slightest agitation

causes clouds of pollen to float lazily away from its dark green heads, if the plant is examined first thing on a still morning.

I have met with one rather remarkable instance of *autogamy* in the case of *Levenhookia pusilla*, R. Br. The plant is very tiny, often scarcely half an inch high. Its pretty pink blossoms are borne in a compact terminal panicle. The corolla measures about one millimetre across. The galeate labellum (fifth petal) closely embraces the erect gynostemium throughout the flower's lifetime unless disturbed. The anthers are mature at anthesis and dehisce within the labellum. A few days afterwards the stigmas are forced up between the anthers by growth of the style apex and receive pollen in the process. In due course fruit matures. That appears to be the usual process. Sometimes, I believe, the labellum is forced off the column by an insect. It seems scarcely possible for one to dip into the blossom without dislodging the labellum as it leaves. Once dislodged the labellum never again embraces the column; so that a flower once visited is dependent upon insect aid for pollination (unless it had been self-pollinated before the visit). I believe the process is exactly similar in *L. stipitata*, F. Muell. In both these species the labellum fits the column very closely, and is quite devoid of irritability. This is of special interest, because in 'Flora Australiensis', vol. iv, p. 34, Bentham states that in contrast with the irritable column of *Stylium*, the labellum is irritable in *Levenhookia*. I have only seen the two species named above. In these loss or lack of irritability has made the labellum an excellent organ for autogamy. This has permitted reduction in size of flower and plant, and made possible the occupation of the arid situations where I have seen it growing.

Throughout this paper I have arranged my examples in inverse order of the *mechanical* complexity of their flowers. I have avoided phylogenetic considerations, which it seems to me could only have tended to obscurity and contention. From the mechanical view-point the flowers of the Proteaceae I have mentioned for simplicity and economy of tissue are a very close approach to perfection, if not, indeed, perfection itself. Yet it is a genus of Myrtaceae—*Eucalyptus*—that forms the dominant vegetation of this country. The perfection of its floral structure is certainly not the only factor governing the measure of a plant's success—often, perhaps, it is of little consequence. There are, however, considerations which suggest that the Proteaceae have over-specialized flowers, their mechanical perfection being a physiological fault, for the simple reason, I suppose, that their pollinators fail to behave in a mechanically perfect manner. Recently I estimated the number of flowers borne and seeds produced by two trees growing within sight of one another. One was a Jarrah (*Eucalyptus marginata*, Sm.) and the other *Banksia attenuata*, R. Br. In round numbers the former bore six hundred and seventy thousand (670,000) flowers and

produced one million seeds, while the latter's sixty-seven thousand flowers produced only four hundred seeds. This surely gives the Eucalypt an enormous advantage over the *Banksia*. It took only seventy-five inflorescences to produce the four hundred *Banksia* seeds, while the Eucalypt required eighty-four thousand to produce its million. The advantage seems not so great; but it must be understood that the *Banksia* inflorescence is enormous compared with that of the Eucalypt. I think these estimates, somewhat rough though they are, fairly represent the advantage in seed production that Eucalypts possess over Grevilleoid Proteaceae. Surely the immensely greater number of its seeds should favour variability in *Eucalyptus*, and even without variation should give it immensely greater power to seize new territory and increase its numbers.

I have been struck by the number of instances I have seen in which several or many small flowers are gathered together into inflorescences resembling, and perhaps often behaving as, one large flower. Its prevalence suggests that there is some special advantage in such an inflorescence. It is seldom, I think, an economy of tissue or a reduction of transpiring surface. A study of the genus *Eucalyptus*, however, suggests an advantage of considerable importance. The large solitary flower is found, for example, in *E. macrocarpa*, and *E. loxophleba*, Benth., will serve for an instance of the anthoidal inflorescence of many small flowers. In the first-named species of 3,000 sq. mm. of flower surface adapted for the disposal or receipt of pollen only the central square millimetre is stigmatic, whereas the inflorescence of *E. loxophleba* presents 12 sq. mm. of stigmatic surface evenly distributed amongst 1,000 sq. mm. of anthers. As there is nothing in the structure of the flowers to compel a pollinator to brush a central stigma with a part of its body which has been pollen-dusted, it seems to me certain that a flower of the latter species has a far better chance of pollination than one of the former, and I think it is not unlikely that it also has a better chance of cross-pollination. I have no exact data; but my experience of the species has given me the impression that the flowers of *E. loxophleba* are far more fertile than those of *E. macrocarpa*. It is possible, however, that failure to fruit is not always due to failure to secure pollination. The solitary-flowered Eucalypts, I believe without exception, are few, well-marked, and restricted in range. The umbel-bearing species are numerous, usually very variable, and they often range over a wide area of territory. It seems possible that the form of inflorescence is contributory to these effects. *Banksia attenuata* has an inflorescence presenting many stigmas well distributed over a very large area, yet it is a very shy seeder. This tends to emphasize the fact that floral structure is but one factor in the production of seed. *Banksia*, however, differs from *Eucalyptus*: its pollen-bearing surface is not very much larger than its stigmatic surface, and its pollen easily rubs off.

## THE ORIGIN OF HONEY-EATING.

Many times, early in the morning, in spring, when a certain Pear-tree is in blossom, I have seen small birds (*Zosterops gouldi*) sipping dewdrops from the leaves of the said tree, and, just occasionally, dipping into a blossom for a sip of sweetness. While in Albany some years ago I saw the same bird taking dewdrops from the foliage of *Adenanthera cuneata*, disdaining apparently the sweeter moisture of the flowers. After a shower a large drop of water remains at the eye of *Templetonia retusa* flowers. These observations seem to me strongly suggestive of the origin of honey-eating in birds. How profoundly what was perhaps an accident—the mistaking of a nectar-drop for a dewdrop—in the far distant past has modified the present face of nature!

## VARIATION.

Three well-marked instances of variation have come under my notice. The labellum of *Caladenia dilata*, R. Br., is usually deeply fringed; but occasionally a flower occurs fringeless. In one locality, Mt. Bakewell, I have never yet found a single specimen with a fringed labellum; all are fringeless. I have seen no intermediates. The change is abrupt and striking. The inner rows of the involucral bracts of *Helichrysum Lawrencella*, F. Muell., are petaloid and usually bright pink. A solitary plant with pure white bracts is occasionally found. On the slopes of Mt. Bakewell white-bracted plants are common, and often form small communities. When such a community is bordered by a pink-bracted one, intermediates of various degrees occur. In this locality pure whites seem the most vigorous. I believe both the above species are frequently self-pollinated, though with insect aid. *Drosera macrantha*, Endl., is usually white flowered, but in one locality I have found pink-flowered plants fairly common, intermixed with the white ones. The pinkness varies in shade from the merest suggestion to pale rose. In the year 1913 thirty-two white-flowered plants produced 39 per cent. of fruits from their flowers, and 36 per cent. of the flowers of sixteen pink-flowered plants were fertile. Selecting several different sixteens from amongst the thirty-two, I found percentages varying between 33 and 48; so that the percentage for the pinks falls well within the limits of observational error. My conclusion is, therefore, that a pink tint to the petals had no selection value that year in the locality in question. In the years 1912 and 1914 I managed to examine a few plants of each variety, and on both occasions the white flowers showed a higher percentage of fertility; so possibly a pink tint is detrimental to these flowers. This raises the question of the value of colour as an attractive agent. All the definite evidence I have collected is negative. Under suitable conditions *Marianthus lineatus* blossoms develop their sweet odour quite strongly; and then they are powerfully

attractive to house-flies. Dried in fine sand the flowers retain their shape and colour perfectly, but they quite lose their attraction for house-flies. This points to the conclusion that their colour is without influence upon those insects. Whether it has any effect upon the indigenous pollinator I do not know. Flowers of every hue are visited by hive-bees apparently with perfect impartiality. Birds also seem quite indifferent to colour. Yearly for some considerable time I have observed cream-coloured flowers (*Dryandra carduacea*), yellow flowers (*Acacia celastrifolia*), and bright red flowers (*Calothamnus sanguineus*) blooming simultaneously in the same locality, so that they compete for the attentions of the same identical birds. Available time has never permitted a serviceable count of visits; but no obvious partiality is shown for either species, and all fruit freely.



# A Comparative List of Fern Pinna-traces, with some Notes on the Leaf-trace in the Ferns.

BY

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With five Figures in the Text.

IN three papers dealing with the anatomy of Fern-leaves<sup>1</sup> the contrast between the 'marginal' and 'extra-marginal' types of pinna-supply from the vascular system of the rachis has been emphasized. The contrast is not perfectly sharp and well defined—in one or two Ferns it is difficult to decide whether the type of pinna-supply is marginal or extra-marginal, for some forms of the extra-marginal type come very close to the marginal. But on the whole it can be said that the same type appears in the species of a genus; that the marginal type is found among the more primitive Ferns in the evolutionary scale and among the most advanced genera; that the extra-marginal type occurs in those Ferns which stand midway in the system of classification and in some advanced forms.

Since the list summarizing the results of investigation up to 1914<sup>2</sup> was published, many Fern-leaves have been examined. The accompanying table shows the full number of species and includes those already mentioned. Every available genus containing members with pinnate leaves has been investigated. For several critical genera additional species have been studied. The list summarizes the result of the examination of 90 genera and 220 species of Ferns. Fifty-one genera with 126 species have the marginal type of pinna-supply; 46 genera with 94 species have the extra-marginal type. The overlap in the numbers of genera is due to the occurrence of both types of pinna-supply in certain genera, viz. *Balanitium*, *Leptochilus*, *Microlepia*, *Odontosoria*, *Coniogramme*, *Notholaena*, and *Onychium*.

The width of the field which I have been able to cover is due to the kindness of the Regius Keeper of the Royal Botanic Garden, Edinburgh,

<sup>1</sup> The Structure and Affinities of *Peranema* and *Diacalpe*, Ann. of Bot., vol. xxvi, pp. 245–68; The Pinna-trace in the Ferns, Trans. Roy. Soc. Edin., vol. i, pp. 349–78; On the Leaf-trace in some Pinnate Leaves, ibid., vol. iii, pp. 1–36.

<sup>2</sup> Trans. Roy. Soc. Edin., vol. i, pp. 349–78.

who has given me free access to the rich collections under his charge. To him and to Professor F. O. Bower, F.R.S., who kindly supplied me with material of several important species, I desire to express my thanks.

In the present list I have been able to group the forms of pinna-supply under two heads. In my last paper<sup>1</sup> I was unable definitely to state that there are only the two types. These two types of pinna-supply differ only in regard to their derivation from the adaxial portion of the leaf-trace; if the portion (or all) of the pinna-trace derived from the adaxial portion of the

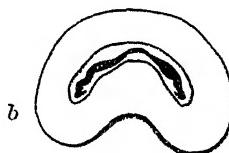
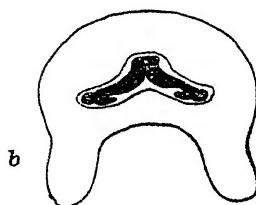
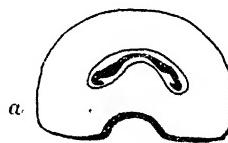
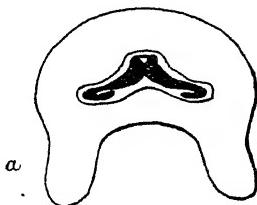


FIG. 1. Diagrams showing the development of the extra-marginal type of pinna-supply in *Dryopteris vivipara* (Raddi), C. Chr.

FIG. 2. Diagrams to illustrate the development of the marginal type of pinna-supply in *Pteris umbrosa*, R. Br.

leaf-trace comes from the back of a hook, we have the 'extra-marginal' type<sup>2</sup> (Fig. 1); if it comes directly from the margin (i. e. if the adaxial margin of the leaf-trace is given off), we have the 'marginal' type<sup>3</sup> (Fig. 2). In either type we may have a portion of the pinna-trace derived from the abaxial side of the leaf-trace. This, a local phenomenon, usually appearing where the pinnae are large, seemed to be the basis of a potential third type

<sup>1</sup> Trans. Roy. Soc. Edin., vol. lii, pp. 1-36.

<sup>2</sup> Ann. of Bot., vol. xxvi, p. 250.

<sup>3</sup> Ibid., p. 251.

of pinna-supply (to which I have referred as the 'combination-type').<sup>1</sup> There is a combination sometimes of an 'extra-marginal' supply with a 'reinforcement' (Fig. 3); sometimes of a 'marginal' supply with a 'reinforcement' (Fig. 4).

These arrangements appear most prominently among the Cyatheaceae, Aspidieae, and Pterideae. Further investigation of the members of the

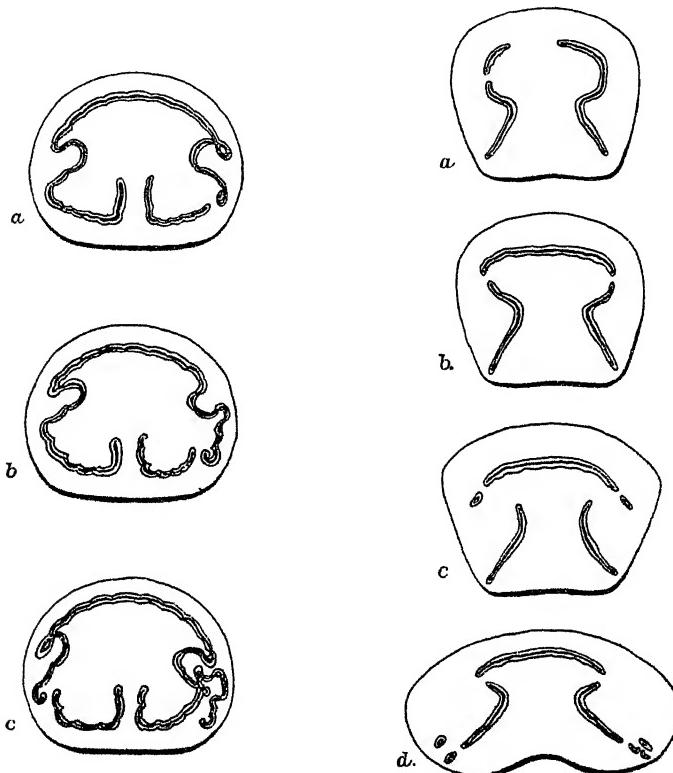


FIG. 3. Diagrams showing the extra-marginal type of pinna-supply, with a 'reinforcement' derived from the abaxial curve of the leaf-trace, in *Cibotium barometz* (L.), J. Sm.

FIG. 4. Diagrams illustrating the marginal type of pinna-supply, with a 'reinforcement' derived from the abaxial curve of the leaf-trace, in *Lonchitis pubescens*, Willd.

Pterideae, especially of the genera *Hypolepis*, *Paesia*, and *Pteris*, has shown that the appearance of the 'reinforcements' is partly dependent on the size of the leaf. For in *Pteris decurrents*, Pr., the marginal supply of the pinnae occurs alone in a fairly small leaf, while the marginal supply is reinforced in a leaf with large pinnae. In the genus *Paesia*, *P. viscosa*, St. Hil., has the marginal supply alone; *P. scaberula* (A. Rich.), Kuhn, possesses a

<sup>1</sup> Trans. Roy. Soc. Edin., vol. lli, pp. 12, 14 (cf. ibid., vol. I, p. 352).

reinforcement in addition to the marginal supply. In *Hypolepis distans* (Col.), Hk., the marginal supply appears alone; in *H. repens* (L.), Pr., and *H. tenuifolia* (Forst.), Bernh., the marginal supply is reinforced.

The example of *Pteris decurrentes* confirms what has been found in species of the genera *Dryopteris*,<sup>1</sup> *Polypodium*,<sup>2</sup> and *Polystichum*,<sup>3</sup> namely, that a 'reinforcement' appears in the pinna-trace where large pinnae are present (Fig. 5). There has been no doubt of the interdependence of the presence of large pinnae and of the appearance of the reinforcement in connexion with leaf-traces composed of several separate vascular strands.

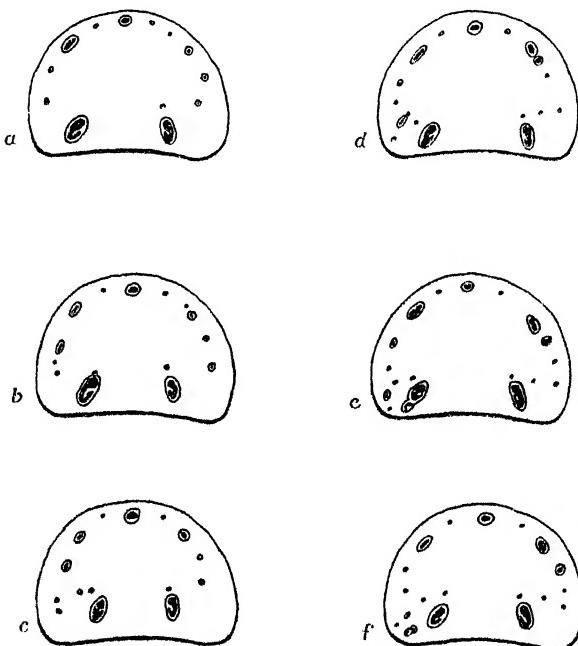


FIG. 5. Diagrams illustrating the extra-marginal type of pinna-supply, with a 'reinforcement' derived from the abaxial curve of the leaf-trace, in *Dryopteris grandis* (Pr.), C. Chr.

This has already been shown in *Polypodium decurrentes*, Raddi,<sup>4</sup> and *Polystichum adiantiforme* (Forst.), J. Sm.<sup>5</sup> We may now add that, even where the leaf-trace is undivided below the first pinnae, the 'reinforcement' of the part of the pinna-trace derived from the adaxial portion of the leaf-trace is connected with the size of the pinnae.

The occurrence of a 'reinforcement' is not to be regarded as helping to constitute a third type of pinna-supply. The two types of pinna-supply already described are fundamentally contrastive; either type may (usually where large pinnae appear) be reinforced from the abaxial portion of the

<sup>1</sup> Trans. Roy. Soc. Edin., vol. lii, p. 11.

<sup>2</sup> Ibid., pp. 7, 12.

<sup>3</sup> Ibid., p. 7.

<sup>4</sup> Ibid., p. 10.

<sup>5</sup> Ibid., p. 10.

leaf-trace from which it departs. In the list on pp. 242-5 the species in which 'reinforcements' have been found are marked with an asterisk.

The nomenclature used in the list is that adopted by Christensen in the 'Index Filicum'. It has already been stated<sup>1</sup> that, with a few exceptions, the same type of pinna-supply is found in the species of a genus, recognized as such in the 'Index Filicum'. Compared with the total number of species named in the 'Index Filicum' (5,940), the number I have examined (220) is very small indeed. But it may be pointed out that members of practically every genus with pinnate-leaved species have been examined. The exceptions among the species in my list are found in seven genera. They would be very much more numerous if other systems of nomenclature (e.g. that given in Hooker and Baker's 'Synopsis Filicum') were adopted. Christensen points out in his preface to the 'Index Filicum' that where possible he has adopted the nomenclature of a monographer of a genus. Such a plan has disadvantages as well as very great advantages. For the standards of specific distinction (or even of generic rank) adopted by the monographer of one genus may be, and most frequently are, very different from those of monographers of other genera. It is significant that the greatest degree of agreement between the arrangement of species in the 'Index Filicum' and that based on the constancy of occurrence of the same type of pinna-supply within a genus is found in the genera *Dryopteris*, *Polystichum*, and *Aspidium*, of which Christensen has himself made a special study. Further, it has been found that frequently where plants placed under certain well-known genera like *Polypodium* or *Polystichum* have a method of pinna-supply different from that prevalent throughout the genus under which they are placed (e.g. in Hooker and Baker's 'Synopsis Filicum'), they are to be found in the 'Index Filicum' under separate generic names, often in genera with only a few species. For example, the species *Phanerophlebia juglandifolia* (H. B. Willd.), J. Sm., placed under *Polypodium* in Hooker and Baker's 'Synopsis Filicum', has the extra-marginal type of pinna-supply; in the species of the genus *Polypodium* recognized in the 'Index Filicum' and enumerated above, the marginal type of pinna-supply prevails. *Polystichum viscidulum*, C. Chr., has the marginal type of pinna-supply; the extra-marginal type of pinna-supply is found throughout the species of *Polystichum* I have examined; in the Addenda of the 'Index Filicum' Christensen adopts Maxon's name and puts this species in the genus *Adenoderris*.

Turning now to the rule of constancy of type of pinna-supply within a genus of Ferns in the 'Index Filicum', we find the exceptions<sup>2</sup> in

*Balantium stramineum* (Labill.), Diels.

*Leptochilus tricuspis* (Hk.), C. Chr.

<sup>1</sup> Trans. Roy. Soc. Edin., vol. lii, p. 15.

<sup>2</sup> Judging the condition for the genus from that shown by the majority of species examined.

- Microlepia hirsuta* (J. Sm.), Pr.  
*Odontosoria aculeata* (L.), J. Sm.  
*Odontosoria chinensis* (L.), J. Sm., var. *Veitchii*.  
*Odontosoria uncinella* (Kze.), Fée.  
*Coniogramme japonica* (Thbg.), Diels.  
*Notholaena marantae* (L.), R. Br.  
*Notholaena sinuata* (Lag.), Klff.  
*Onychium japonicum* (Thbg.), Kze.

It is noticeable that none of these species retain the generic names given them when they were first described. In some of the genera the species which have been examined have been named by different authors. This is not the case for all, however, for the three species of *Leptochilus* in the list on p. 243 have been named by Christensen (whose determinations in the genera *Dryopteris*, *Polystichum*, and *Aspidium* agree so closely with the grouping based on the type of pinna-supply found in the species), and three out of five of the species of *Onychium*, including the divergent *O. japonicum*, have been named by Otto Kunze.

In some of the ten species named above, the divergence in the type of pinna-supply is paralleled by divergences in other features. *Microlepia hirsuta*, for example, has been shown by Professor Bower<sup>1</sup> to stand aloof from other species of *Microlepia* in its soral and sporangial characters. *Balantium stramineum* has been placed in the genera *Dicksonia* (Labill.), *Sitolobium* (Brack.), and *Dennstaedtia* (J. Sm.). In *Balantium culcita* (L'Hérit.), Klff., and *B. coniifolium* (Hk.), J. Sm., the marginal type of pinna-supply is found; the extra-marginal type occurs in *B. stramineum* and in the species examined of *Dicksonia*, *Sitolobium*, and *Dennstaedtia*. *Leptochilus tricuspis* has the marginal type of pinna-supply; the extra-marginal type is found in other species of the genus. Professor Bower has recently shown<sup>2</sup> that *L. tricuspis* differs in its 'diplodesmic structure' and in its 'advanced perforation of the solenosteles' from other species of the genus. He separates this species as a member of a monotypic genus, under the name *Gymnopteris tricuspis* (Hook.), Bedd. *Odontosoria aculeata* (L.), J. Sm., *O. chinensis* (L.), J. Sm., var. *Veitchii*, *O. uncinella* (Kze.), Fée, were placed with the species of *Microlepia* by Mettenius in 1856; *O. bifida* (Klf.), J. Sm., *O. clavata* (L.), J. Sm., *O. meifolia* (H. B. K.), C. Chr., *O. retusa* (Cav.), J. Sm., have been placed in various genera, but not in *Microlepia*. *O. aculeata*, *O. chinensis*, var. *Veitchii*, and *O. uncinella* have, like the species of *Microlepia*, the extra-marginal type of pinna-supply; the other species have the marginal type. The generic name *Coniogramme* was proposed by Fée for several species now reduced to *C. fraxinea* (Don), Diels. *C. japonica* (Thbg.), Diels, has been variously assigned to *Hemionitis* (Thbg.), *Gymnogramma* (Desv.), *Dictyogramme* ([Pr.], Fée), and *Notogramme* (Pr.).

<sup>1</sup> Ann. of Bot., vol. xvii, p. 732.<sup>2</sup> Ibid., vol. xxxi, p. 36.

under none of which has *C. fraxinea* been placed. Both species were placed in *Gymnogramme* (but in different sections of the genus) in Hooker and Baker's 'Synopsis Filicum'. Fée, at the time of proposing the generic name for *Coniogramme fraxinea*, proposed the name *Dictyogramme* for Diels' *C. japonica*. This separation is certainly upheld from the result of examination of the pinna-traces of the two species. *Notholaena affinis* (Mett.), Moore, *N. bonariensis* (Willd.), C. Chr., *N. distans*, R. Br., *N. hirsuta* (Poir.), Desv., *N. hypoleuca*, Kze., and *N. mollis*, Kze., have all been placed in the genus *Cheilanthes* by Mettenius; they agree in having the marginal type of pinna-supply. *Notholaena marantae* and *N. sinuata*, which have the extra-marginal type of pinna-supply, have been placed in various genera, but never in *Cheilanthes*. In the genus *Onychium* all four species examined have regularly been grouped together (by Prantl—in *Cryptogramme*—by Hooker and Baker in the 'Synopsis Filicum', and by Christ in 'Die Farnkräuter der Erde'). There is in this genus no explanation from the nomenclatural data which suggests an isolation of *O. japonicum* from the other species.

In these notes on the 'aberrant' species I by no means wish to suggest a re-grouping based on their types of pinna-supply. The rule regarding the constancy of occurrence of one type of pinna-supply within a genus is not invariably applicable, yet in so large a number of the species examined does it apply, and in several critical genera so closely does a grouping based on the type of pinna-supply correspond with the grouping of species made on other grounds, while so many of the exceptions to the rule have been more or less problematical to systematists, that it would seem that the rule is founded on some real phenomenon and not on mere chance. It is the marked constancy of occurrence of one type of pinna-supply within a genus of Ferns which makes it appear probable that changes in those structural features of a Fern, customarily used for purposes of systematic classification (including generic delimitation), have been accompanied by alterations in the form and system of branching of the leaf-trace. These alterations are represented, in part at least, by the abandonment of one type of pinna-supply and the adoption of the other. It was this constancy which led me to use<sup>1</sup> the attractively concise, if not legitimate, expression, that 'systematic position' is one of 'the factors which control the form of leaf-trace and its system of branching'.

It may be noted that while the marginal or extra-marginal type of pinna-supply has been recorded as characteristic of certain species, the same type of pinna-supply is not found in connexion with *every* pinna of any Fern-leaf. In many leaves—indeed, in the majority examined—the ultimate pinnae,<sup>2</sup> and frequently several pairs below the tip of a leaf,<sup>3</sup> are

<sup>1</sup> Trans. Roy. Soc. Edin., vol. lii, pp. 24, 32.

<sup>2</sup> Ann. of Bot., vol. xxvi, p. 251.

<sup>3</sup> Trans. Roy. Soc. Edin., vol. i, p. 360.

supplied with their pinna-systems on the marginal plan. But wherever the extra-marginal type of pinna-supply has been found in connexion with any of the pinnae of a leaf, it has also been found that the basal pinnae are supplied extra-marginally. In a paper recently published<sup>1</sup> from the notes of the late Professor Gwynne-Vaughan, it is remarked that 'it must be remembered that in some species the lowest branches of the rachis and of the primary branches are themselves reduced in size, being markedly smaller than some of those higher up. This reduction affects the method of branching so that it may present features of a more or less primitive type.' In the species which I have examined, several plants have had basal pinnae smaller than those higher up the leaf, but, though there sometimes is a marked reduction in the size of the pinna-trace for these pinnae, compared with that of the pinna-traces of the higher branches, no actual divergence from the type prevalent throughout the lower pinnae has been found. Dr. J. M. Thompson tells me, however, that he has found an irregular occurrence of the extra-marginal type of pinna-supply in leaves of *Trismeria trifoliata* (L.), Diels—sometimes the extra-marginal type is found, sometimes the marginal. And the one may succeed the other and in turn be succeeded by it as one passes up the leaf from pinna to pinna. In the leaves of *Trismeria trifoliata*, which I have examined, the extra-marginal type certainly occurs regularly throughout the length of the leaf.

We find, too, that in Ferns which have the extra-marginal type of pinna-supply in the fully mature leaves, the marginal type is regularly found in the earliest leaves.<sup>2</sup> In order, therefore, to make use of the criterion of the type of pinna-supply, we must examine the lower pinnae of the older leaves of the Ferns we wish to compare.

The results of the investigations detailed in the three earlier papers and in this may now be summarized.

The amount of xylem in the adaxial portion of the leaf-trace is dependent on the situation in which the Fern grows;<sup>3</sup> the abaxial complications depend on the length of the leaf;<sup>4</sup> the reinforcement of the adaxial strands, on the close crowding together of the pinnae;<sup>5</sup> the reinforcement of the portion of the pinna-trace derived from the adaxial side of the leaf-trace, on the size and complexity of the pinnae.<sup>6</sup>

The presence of adaxial hooks in the leaf-trace seems in great measure to depend on a factor connected with heredity; the hooks may have appeared as Fern-leaves increased in size.<sup>7</sup>

The form of the pinna-trace depends (1) on the presence or absence of hooks in the leaf-trace (an inherited feature—useful, therefore, in phylo-

<sup>1</sup> Ann. of Bot., vol. xxx, p. 491.

<sup>2</sup> Trans. Roy. Soc. Edin., vol. 1, pp. 360, 361.

<sup>3</sup> Ibid., vol. lii, p. 4.

<sup>4</sup> Ibid., pp. 5, 6, 8, 12.

<sup>5</sup> Ibid., pp. 6, 7.

<sup>6</sup> Ibid., pp. 7, 9, 10, 11, 13, 14; above, p. 236.

<sup>7</sup> Cf. A. G. Tansley, Ev. of Fil. Vasc. System, p. 117.

geny); (2) on the size of the pinnae (a local and individual feature—of practically no value in phylogeny).

The adaxial portion of the pinna-trace is the portion dependent on heredity; the abaxial portion is variable in relation to features of the individual leaf.

The form of the adaxial side of the leaf-trace is connected with the phylogeny of the Fern in which it occurs; its outline and relations are useful as phylogenetic criteria. The form of the abaxial portion is dependent upon individual peculiarities,<sup>1</sup> and is therefore of little value in phylogenetic criticism.

The way in which the adaxial part of the leaf-trace gives off part (or all) of the pinna-trace is important, as that part of the leaf-trace alters its form apparently in response to phylogenetic influences. The actual shape of the pinna-trace is of little importance, but the method of development of the portion of it which comes from the adaxial side of the leaf-trace appears likely to be a useful addition to the criteria of value in the study of the phylogeny of the Ferns.

#### SUMMARY.

1. There are two types of pinna-supply from the leaf-trace in the Ferns. In the 'extra-marginal' type, the portion of the pinna-trace which comes from the adaxial side of the leaf-trace is nipped off from the back of a 'hook', technically from the abaxial face of the curved leaf-trace; the extreme tip of the adaxial portion of the leaf-trace is continued upward as part of the leaf-trace. In the 'marginal' type, the adaxial portion of the leaf-trace (nearest to the pinna) is itself given off to supply the pinna. In both types (usually in connexion with large pinnae) a portion of the pinna-trace may be derived from the abaxial side of the leaf-trace.

2. A tabular scheme of the distribution of the two types of pinna-supply in the Ferns has been drawn up; among the Ferns examined, the extra-marginal type occurs in 46 genera with 94 species, the marginal type is found in 51 genera with 126 species.

3. With a few exceptions (seven genera), the rule holds that the same type of pinna-supply is found in the species of a genus of Ferns recognized as such in Christensen's 'Index Filicum'. Even in the exceptions, there is considerable agreement between the grouping of the species according to

<sup>1</sup> Among entire-leaved species of the genus *Polypodium* there have been examined *P. vaccinifolium*, Langsd. et Fisch.; *P. lycopodioides*, Linn.; *P. percussum*, Cav.; *P. pustulatum*, Forst.; *P. glaucophyllum*, Kze.; *P. Xiphias* (Moore), Bak.; *P. lingua*, Vahl; *P. crassifolium*, Linn.; and *P. punctatum* (L.), Sw. In *P. vaccinifolium*, *P. lycopodioides*, and *P. percussum* the leaf is short; only one or two strands regularly occur in the leaf-trace. In *P. pustulatum*, *P. glaucophyllum*, *P. Xiphias*, and *P. lingua* the leaves are longer; strands appear on the abaxial side of the leaf-trace, in addition to the adaxial pair. In *P. crassifolium* and *P. punctatum*, which have leaves longer than those of any of the preceding species, there is a strongly developed abaxial system of many strands.

the type of pinna-supply found in them and that made on other grounds by some systematists.

4. In order to make use of the criterion of the type of pinna-supply in the Ferns, the lower pinnae of the older leaves must be examined.

Family.	Marginal Type of Pinna-supply.	Extra-marginal Type of Pinna-supply.
Osmundaceae		<i>Todea barbara</i> (L.), Moore. <i>Leptopteris Fraseri</i> (Hk. and Grev.), Pr.; <i>L. hymenophylloides</i> (A. Rich.), Pr. <i>Osmunda juvanica</i> , Bl.; <i>O. regalis</i> , L.
Schizaeaceae	<i>Lygodium circinnatum</i> (Buism.), Sw.; <i>L. scandens</i> (L.), Sw. <i>Mohria caffrorum</i> (L.), Desv. <i>Anemia collina</i> , Raddi; <i>A. hirta</i> (L.), Sw.; <i>A. phyllitidis</i> (L.), Sw.; <i>A. rotundifolia</i> , Schrad.	
Gleicheniaceae		<i>Gleichenia circinnata</i> , Sw., var. <i>speciosa</i> ; <i>G. flabellata</i> , R. Br.; <i>G. rupestris</i> , R. Br.
Hymenophyllaceae	<i>Loxoma Cunninghamii</i> , R. Br.	<i>Hymenophyllum demissum</i> (Forst.), Sw.; <i>H. dilatatum</i> (Forst.), Sw. <i>Trichomanes elegans</i> , Rich.; <i>T. radicans</i> , Sw.
Dicksoniaceae	<i>Balantium culcita</i> (L'Hérit.), Kl.; <i>B. coniifolium</i> (Hk.), J. Sm.	<i>Balantium stramineum</i> (Labill.), Diels. <i>Dicksonia antarctica</i> , Lab.; <i>D. fibrosa</i> , Col. <sup>1</sup> <i>*Cibotium barometz</i> (L.), J. Sm.; <sup>2</sup> <i>C. regale</i> , Linden; <i>C. Schiedei</i> , Schlecht. et Cham. <i>C. Wendlandii</i> , Mett., var. <i>Vershafeltii</i> .
Thelypteridaceae		<i>*Thelypteris elegans</i> , Kze.
Cyatheae		<i>*Cyathea mexicana</i> , Schlecht. et Cham.; <i>*C. pubescens</i> , Mett. <i>*Hemitelia grandifolia</i> (Willd.), Spr. <i>*Alsophila glauca</i> (Bl.), J. Sm.
Woodsieae (Woodsiinae)	<i>Cystopteris fragilis</i> (L.), Bernh.; <i>C. montana</i> (Lam.), Bernh. <i>*Hypoderris heteroneurooides</i> , Christ.	<i>Diacalpe aspidioides</i> , Bl. <i>Peranema cyatheoides</i> , Don. <i>Woodisia ilvensis</i> (L.), R. Br.; <i>W. polystichoides</i> , Eat. <i>Acrophorus stipellatus</i> (Wall.), Moore.

<sup>1</sup> In Trans. Roy. Soc. Edin., vol. I, p. 354, this species was described as having the marginal type of pinna-supply. The identification of the material then examined must have been incorrect. Properly authenticated material since examined shows this species to have the extra-marginal type of pinna-supply.

<sup>2</sup> The species marked with an asterisk are those in which 'reinforcements' have been found (see pp. 236, 237).

Family.	Marginal Type of Pinna-supply.	Extra-marginal Type of Pinna-supply.
(Onocleineae)		<i>Matteuccia orientalis</i> (Hk.), Trev.; <i>M. struthiopteris</i> (L.), Todaro. <i>Onoclea sensibilis</i> , L.
Aspidieae (Aspidiinae)	<i>*Aspidium martinicense</i> , Spr. ; <i>*A. trifoliatum</i> (L.), Sw. <i>Adenoderris viscidula</i> (Mett.), Maxon. <i>*Cyclodium meniscioides</i> (Willd.), Pr. <i>*Polybotrya cervina</i> (L.), Kl. <i>*Leptochilus tricuspis</i> (Hk.), C. Chr.	<i>Dryopteris filix-mas</i> (L.), Schott ; <i>*D. grandis</i> (Pr.), C. Chr. ; <i>D. phlegopteris</i> (L.), C. Chr. ; <i>D. pustulifera</i> (Bedd.), O. Ktze. ; <i>D. serrata</i> (Cav.), C. Chr. ; <i>D. setigera</i> (Bl.), O. Ktze. ; <i>D. vivipara</i> (Raddi), C. Chr. <i>Mesochlaena polycarpa</i> (Bl.), Bedd. <i>Didymochlaena truncatula</i> (Sw.), J. Sm. <i>Cyclopeltis semicordata</i> (Sw.), J. Sm. <i>Polystichum aculeatum</i> (L.), Schott, var. <i>angulare</i> , Pr. ; <i>*P. adiantiforme</i> (Forst.), J. Sm. ; <i>P. falcatum</i> (L. fil.), Diels ; <i>P. Hookerianum</i> (Pr.), C. Chr. ; <i>P. Standishii</i> (Moore), C. Chr. ; <i>P. vestitum</i> (Forst.), Pr. <i>Phanerophlebia juglandifolia</i> , (H. B. Willd.), J. Sm. <i>Plecosorus speciosissimus</i> (A. Br.), Moore. <i>*Leptochilus cuspidatus</i> (Pr.), C. Chr. ; <i>*L. guianensis</i> (Aublet), C. Chr.
Davallieae	<i>Davallia assamica</i> (Bedd.), Bak. ; <i>D. bullata</i> , Wall. ; <i>D. dissecta</i> , J. Sm. ; <i>D. immersa</i> , Wall. ; <i>D. pallida</i> , Mett. ; <i>D. pentaphylla</i> , Bl. ; <i>D. solida</i> (Forst.), Sw. ; <i>D. solida</i> , var. <i>sibirica</i> . <i>Arthropteris altescandens</i> (Colla), J. Sm. <i>Nephrolepis Amerpohlii</i> , hort. ; <i>N. Posteri</i> , hort., Hill ; <i>N. Piersoni</i> , hort. ; <i>N. Scottii</i> , hort. <i>Humata botrychoides</i> , Brack. ; <i>H. repens</i> (L. fil.), Diels, var. <i>alpina</i> ; <i>H. vestita</i> (Bl.), Moore ; <i>*Saccoloma domingense</i> (Spr.), Prantl ; <i>S. Imrayanum</i> , Hook. <i>Diellia falcata</i> , Brack. ; <i>D. pumila</i> , Brack. <i>Microlepia hirsuta</i> (J. Sm.), Pr. <i>Lindsaya repens</i> (Bory), Bedd. ; <i>L. stricta</i> (Sw.), Dry. <i>Odontosoria bifida</i> (Klf.), J. Sm. ; <i>O. clavata</i> (L.), J. Sm. ; <i>O. meifolia</i> (H. B. K.), C. Chr. ; <i>O. retusa</i> (Cav.), J. Sm. <i>Tapeinidium Denhami</i> (Hk.), C. Chr. ; <i>T. pinnatum</i> (Cav.), C. Chr. <i>Schizoloma ensifolium</i> (Sw.), J. Sm.	<i>Leptolepia novae-zelandiae</i> (Col.), Kuhn. <i>Microlepia hirta</i> (Klf.), Pr. ; <i>M. hirta</i> , var. <i>cristata</i> ; <i>M. platyphylla</i> (Don), J. Sm. ; <i>M. speluncar</i> (L.), Moore ; <i>M. strigosa</i> (Thbg.), Pr. <i>Odontosoria aculeata</i> (L.), J. Sm. ; <i>O. chinensis</i> (L.), J. Sm., var. <i>Veitchii</i> ; <i>O. uncinella</i> (Kze.), Fée. <i>Dennstaedtia adiantoides</i> (H. B. Willd.), Moore. <i>Monachosorum subdigitatum</i> (Sw.), J. Sm.

Family.	Marginal Type of Pinna-supply.	Extra-marginal Type of Pinna-supply.
Asplenieae (Aspleniinae)	<i>Asplenium adiantum nigrum</i> , L. ; <i>A. bulbiferum</i> , Forst., var. <i>Fabianum</i> ; <i>A. bulbiferum</i> , var. <i>Hillii</i> ; <i>A. Findleysonianum</i> , Wall. ; <i>A. obtusatum</i> , Forst. ; <i>A. pinnatifidum</i> , Nutt. ; <i>A. praemorsum</i> , Sw. ; <i>A. ruta muraria</i> , L. ; <i>A. tenerum</i> , Forst. ; <i>A. trichomanes</i> , L. <i>Ceterach aureum</i> (Cav.), L. v. Buch. ; <i>C. Dalhousiae</i> (Hk.), C. Chr. ; <i>C. officinarum</i> , DC.	<i>Athyrium filix foemina</i> (L.), Roth ; <i>A. alpestre</i> (Hoppe), Rylands. <i>Diplazium celtidifolium</i> , Kze. ; <i>D. marginatum</i> (L.), Diels ; <i>D. Shepherdii</i> (Spr.), Link. <i>Diplaziopsis javanica</i> (Bl.), C. Chr.
(Blechninae)		<i>Blechnum attenuatum</i> (Sw.), Mett. ; <i>B. Banksii</i> (Hk. fil.), Mett. ; <i>B. brasiliense</i> , Desv. ; <i>B. capense</i> (L.), Schlecht. ; <i>B. discolor</i> (Forst.), Keys. ; <i>B. lanceolatum</i> (R. Br.), Sturm. ; <i>B. Moorei</i> , C. Chr. ; <i>B. occidentale</i> , Mett. ; <i>B. orientale</i> , L. ; <i>B. Patersoni</i> (R. Br.), Mett. ; <i>B. punctulatum</i> , Sw., var. <i>Krebsii</i> , Kze. ; <i>B. spirant</i> (L.), Wither. ; <i>B. tabulare</i> (Thbg.), Kuhn. <i>*Sadleria cyatheoides</i> , Klf. <i>Brainea insignis</i> (Hk.), J. Sm. <i>*Stenochlaena sorbifolia</i> (L.), J. Sm. <i>Woodwardia radicans</i> (L.), Sm. <i>Doodia aspera</i> , R. Br., var. <i>multifida</i> .
Pterideae (Gymnogramminae)	<i>Anogramma leptophylla</i> (L.), Link. <i>Gymnogramma Pearcei</i> , Moore, var. <i>robusta</i> . <i>Coniogramme fraxinea</i> (Don), Diels. <i>Gymnopteris tomentosa</i> (Lam.), Und. <i>Ceropteris calomelanos</i> (L.), Und. ; <i>C. calomelanos</i> , var. <i>chrysophylla</i> , Klfl.	<i>Coniogramme japonica</i> (Thbg.), Diels. <i>Trismeria trifoliata</i> (L.), Diels.
(Cheilanthinae)	<i>Pellaea auriculata</i> (Thbg.), Fée ; <i>P. hastata</i> (Thbg.), Prantl ; <i>P. nivea</i> (Poir.), Prantl ; <i>P. rotundifolia</i> (Forst.), Hk. <i>Notholaena affinis</i> (Mett.), Moore ; <i>N. bonariensis</i> (Willd.), C. Chr. ; <i>N. distans</i> , R. Br. ; <i>N. hirsuta</i> (Poir.), Desv. ; <i>N. hypoleuca</i> , Kze. ; <i>N. mollis</i> , Kze. <i>Cheilanthes argentea</i> (Gmel.), Kze. ; <i>C. myriophylla</i> , Desv., var. <i>elegans</i> . <i>Hypolepis distans</i> (Col.), Hk. ; <i>*H. repens</i> (L.), Pr. ; <i>*H. tenuifolia</i> (Forst.), Bernh. <i>Llavea cordifolia</i> , Lag. <i>Onychium melanolepis</i> (Dene.), Kze. ; <i>O. siliculosum</i> (Desv.), C. Chr. ; <i>O. strictum</i> , Kze. <i>Cryptogramma crispa</i> (L.), R. Br. ; <i>C. Stelleri</i> (Gmel.), Prantl. <i>Plagiozygia glauca</i> (Bl.), Mett.	<i>Notholaena Marantae</i> (L.), R. Br. ; <i>N. sinuata</i> (Lag.), Klfl. <i>Onychium japonicum</i> (Thbg.), Kze.

Family.	Marginal Type of Pinna-supply.	Extra-marginal Type of Pinna-supply.
(Adiantinae)		<i>Adiantum polyphyllum</i> , Willd.; <i>A. sanctae Catharinae</i> , hort., J. Sm.
(Pteridinae)	<i>Actiniopteris australis</i> (L. fil.), Link. <i>Anopterus hexagona</i> (L.), C. Chr. *iOchropteris pallens (Sw.), J. Sm. <i>Pteris atrovirens</i> , Willd.; <i>P. biurita</i> , L.; <i>P. cretica</i> , L.; <i>P. decurrenti</i> , Pr.; <i>P. macilenta</i> , A. Rich.; <i>P. podophylla</i> , Sw.; <i>P. tremula</i> , R. Br.; <i>P. umbrosa</i> , R. Br. *iHistiopteris incisa (Thbg.), J. Sm. *iPteridium aquilinum (L.), Kuhn. *iPuesia scaberula (A. Rich.), Kuhn; <i>P. viscosa</i> , St. Hil. <i>Lonchitis hirsuta</i> , L.; *iL. pubescens, Willd.	
Polypodiaceae (Taenitidinae)	<i>Taenitis blechnoides</i> (Willd.), Sw.	
(Polypodiinae)	<i>Polypodium aureum</i> , L.; <i>P. brasiliense</i> , Poir.; <i>P. Catharinæ</i> , Langsd. et Fisch.; <i>P. cultissimum</i> , Willd.; *iP. decurrens, Raddi; <i>P. ellipticum</i> , Thbg.; *iP. Fendleri, Eat.; <i>P. lepidopteris</i> (Langsd. et Fisch.), Kze.; <i>P. loricatum</i> , L.; <i>P. phymatodes</i> , L.; <i>P. plumula</i> , H. B. Willd.; <i>P. polypodioides</i> (L.), Hitchcock; <i>P. Schneideri</i> , hort., Veitch; <i>P. serrulatum</i> (Sw.), Mett.; <i>P. vulgare</i> , L. <i>Dryostachyum drynarioides</i> (Hk.), Kuhn. <i>Drynaria rigidula</i> (Sw.), Bedd.	
Acrosticheae (Acrostichinae)	<i>Trachypteris pinnata</i> (Hk. fil.), C. Chr. (fertile leaf).	



## The Laticiferous System of *Hevea brasiliensis* and its Protective Function.

BY

A. SHARPLES.

EXPERIMENTAL work designed to test the suggestions put forward to explain the significance of the rôle played by the laticiferous system of latex-bearing trees has seldom been attempted. Of the suggestions long standing, the one that latex exercised a protective function against fungus and insect attacks has received popular acceptance amongst the rubber-planting community of the Middle East. M. George Vernet brought forward evidence indicating that small boring-beetles, e.g. *Xyleborus parvulus*, *Ptorelephia melaneura*, could penetrate the bark of a healthy rubber tree without being killed by the latex, but the general opinion was stated by Pratt:<sup>1</sup> 'In most cases of insect attack' (with special reference to shot-hole borer [*Xyleborus parvulus*]) 'on *Hevea brasiliensis* which have been personally observed it would appear that a fungus disease preceded the insect attack.' Thus it would appear that a healthy tree with a sound laticiferous system could not be penetrated by insects; the latter are only capable of penetrating into the wood when the laticiferous cells are killed previously by the fungus hyphae.

The method of tapping *Hevea brasiliensis* and the wound-response exhibited is universally known and needs no further description. Many methods of artificially stimulating this wound-response and so obtaining a correspondingly greater yield of latex have been suggested and tried on the plantations. One method, recently given much publicity and tried on a large scale, was suggested by Dr. Fickendy, late Director of Agriculture in Samoa. He applied for patent rights, and claimed an increase yield of 50 per cent:

His method consisted of scraping the bark over the proposed tapping area some time previous to the tapping. When the outer corky bark is gently scraped away with a blunt instrument, the cork-cambium (phellogen) is exposed as a green layer overlying the cortical cells. Fickendy's suggestion was to scrape away the corky cells, exposing the phellogen, but leaving the latter intact. The response to the stimulus of scraping should be a substantial increase in the yield of latex.

<sup>1</sup> Pratt, H. C.: Report of Director of Agriculture, F.M.S., 1914.

One rubber estate in Malaya adopted Fickendy's methods and soon began to suffer from attacks by *Xyleborus parvulus* (shot-hole borer). When the attacked trees were inspected it appeared that the bark-scraping was the fundamental cause of the trouble. The explanation was unsatisfactory in the absence of experimental data, so an experiment was started to test the effect of bark-scraping upon rubber trees, with a view to elucidating whether such treatment makes the trees more liable to attack from fungi and insects. For the experiment 30 well-grown, 5 years old, untapped trees were taken. They were divided into five blocks, each containing six trees. The blocks were marked 1 A, 1 B, 1 C, 2 A, and 2 B. Block 1 C acted as control to Blocks 1 A and 1 B. The treated trees were scraped on 28/5/15, over half their circumference, to a height of four feet from the ground. Care was taken that the laticiferous layer was unharmed, no latex exuding after the scraping.

*Block 1 A.* Scraped heavily, the green cork-cambium being removed. The scraped area was watered daily, with a hand-pump, for a few minutes.

*Block 1 B.* Trees scraped lightly, the green cork-cambium (phellogen) being left intact. Watered as 1 A.

*Block 1 C.* Unscraped, but water applied as 1 A and 1 B.

*Block 2 A.* Scraped as 1 A, but no water applied over scraped areas.

*Block 2 B.* Scraped as 1 B, but not watered.

Blocks 1 A, 1 B, and 1 C were treated with water daily in order to prevent a spell of dry weather interfering with the progress of the experiment. The results given below of the treatment are most interesting. A daily examination of the trees was made, the dates given below indicating the day upon which the attacked trees were first noticed.

*Examined 1/6/15.* Block 1 A showed one tree badly attacked by boring beetles, the latex streaming down the bark. The remaining five trees in this block also showed attempts on the part of the insects to pierce the bark. None of the trees of the other blocks showed any sign of attack.

*Examined 6/6/15.* One tree in Block 2 A very badly attacked by borers.

*Examined 11/6/15.* One tree in Block 1 B attacked by borers; another tree in Block 2 A showed signs of a bursting of the bark, from which latex was exuding.

*Examined 14/6/15.* A third tree in Block 2 A showed signs of the bark bursting, with exudation of latex.

*Examined 30/6/15.* One tree in Block 2 A shows cracks in bark with latex exuding.

From 28/5/15 to 2/6/15, there was no rain; from 2/6/15, daily showers were the rule.

Thus, out of twenty-four treated trees, five were attacked as a result of the bark-scraping. Of these, four were heavily scraped and only one lightly

scraped. The control trees in Block 1 C and the remaining untreated trees in the surrounding area were unaffected. The unscraped areas of the treated trees showed no sign of beetle attack.

The trees were affected in two different ways: One tree in Block 1 A, one in Block 1 B, and one in Block 2 A, suffered from a direct attack from boring beetles. The bark of the remaining two trees affected in Block 2 A were attacked by a fungus, which progressed rapidly in one case, killing the bark over a large area, but in the other only small areas of bark were attacked, and this tree now showed signs of recovery. The tree with cracks in the bark and latex exuding in Block 2 B was not affected further. A striking feature was the rapidity of the attack, borers being active on the first affected tree within four days.

The results show that removal of the outer corky layers increases the susceptibility of the trees to attack by fungi and insects. They show also that if the green cork-cambium is left intact the susceptibility to attack is less than it is when this layer is scraped away. This experiment indicates that the corky integument is the important protective layer against insect and fungus attacks and not the laticiferous layer. The activity of the borers on the trees in Block 1 A and 2 A, within a week of scraping the bark caused exudation of the latex in streams down the scraped areas. Dozens of beetles were caught in the streaming latex, but this did not prevent others getting through the bark into the wood. It may be emphasized here, that the experimental plot is clear of timber, is fairly widely planted, 24 ft. by 12 ft., and more or less isolated from the rest of the plantations, so that conditions are not favourable for the insects. The rapid nature of the attack prevents the assumption of a fungus preceding and killing the tissue in advance of the borers.

The results obtained confirm field observations. The years 1915-16 have been noteworthy for a very heavy wintering of the trees on the plantations during the months January, February, and March. The wintering period in both cases coincided with a spell of dry weather and numerous cases of leaf fires occurred, the thick carpet of leaves on the ground burning furiously. The trunks of the trees in the area through which the fires passed were scorched up to a height of ten feet, and borers were quickly at work on the scorched portions of the trees. In every case a copious exudation of latex was noticeable from the bore-holes, but this did not prevent the entry of the insects. One estate was visited four days after the fires had been put out, and the boring beetles were actively working on half a dozen trees not badly scorched and from which there was a good flow of latex beneath the scorched areas. The laticiferous system was obviously healthy at the time of the attack, but the flowing latex did not prevent the insects penetrating into the wood. If the outer corky, protective layers of *Hevea brasiliensis* are removed or injured the tree is liable to a direct attack from boring beetles.

Heavy scraping of the bark, i. e. removal of the cork-cambium, exposes the outer old cortical cells. It is possible that in such tissues a cork-cambial layer does not form very readily, so that the outer cortical cells are exposed to the atmosphere for a comparatively long period. If this coincides with a period of wet weather, the exposed cells become water-logged and their activities interfered with. As a result, numerous ordinarily saprophytic fungi flourish on the exterior and grow inwards gradually, rotting the bark from the outside. Undoubtedly this is the method of attack in cases where there is not a direct attack by borers. The same applies to trees scraped so as to leave the cork-forming cambium intact. Such trees are not so readily attacked, due to the active cork-cambium quickly cutting off cork cells to the outside, forming a new protective layer. This rapid formation of cork prevents water-logging of the cells, and the danger of attack is considerably lessened thereby. But if the cork-cambium cells by any chance become water-logged, their activities are interfered with, and in such a case the bark is as liable to attack as that of the heavily scraped trees.

During tapping operations the inner cortical tissues are opened up. In this connexion, the danger of bark affections similar to those observed in the experiment is slight. There is a considerable difference between exposing outer cortical tissues by scraping, and inner cortical tissues by paring, as in tapping. When tapping, the tissues down to the wood and bast-forming cambium are removed. Thus only very narrow strips of actively dividing cells are exposed at any one time. The coagulated latex along the tapping cuts protects these exposed strips to some extent, while the actively dividing cambium quickly cuts off layers of cells, one of which takes on the functions of a cork-forming cambium and the protective layer is soon replaced.

The selective nature of the borer attack was a noticeable feature. On Block I A, two days after scraping, signs of borers could be observed on every tree. Later the insects appeared to concentrate in a vigorous attack upon one tree. Such selection is difficult to explain, but is probably associated with the fact that plantation conditions are fundamentally unhealthy. Our conception of a healthy tree is limited by a spreading crown of leaves and roots apparently unattacked by disease. This gives no indication of one tree being more susceptible to disease than another, yet such is the case. Under forest conditions the struggle for existence operates in a drastic manner, and susceptible individuals seldom enter the competition. Plantation conditions considerably modify the struggle, and there must be large numbers of apparently healthy trees surviving which under natural conditions could never reach maturity.

Fickendy, in this application for patent rights, says that 'the bark should be scraped down to the cork-forming cambium; leaving the latter intact'. The experimental results show that the trees carefully treated as

above may be attacked by borers and fungi, but that the susceptibility to attack is enormously increased if the cork-cambium is removed. The scraping of the bark so as to leave the cork-cambium intact, without exposing the outer cortical tissues, is a practical impossibility under estate conditions. This layer is very thin, and as the scraping would have to be performed by coolies, there is not the slightest doubt that large patches of the cork-forming layer would be removed. Such trees are comparable to the heavily scraped trees in the experiment.

It follows that bark-scraping of any description should be carried out with discretion. It is quite easy, with a little care, to scrape off the scaly bark on old trees without injury. This is done on large numbers of estates to ensure a clean yield of latex. It has been noticed on several estates that this has been undertaken so carelessly as to expose the outer cortical tissues, thereby laying the trees open to injury. Such carelessness must be severely condemned, and estates managers would be well repaid if they inspected scraped trees immediately after the scraping.

To all patent methods of increasing yield of latex there are serious objections. It is admitted by authorities that it is possible to exhaust a rubber tree by draining it too rapidly of its latex. This conclusion has been reached as a result of practical experience. Unfortunately there is little positive knowledge as to the function of latex in the economy of the tree, and until we gain more knowledge it is necessary to urge the desirability of a conservative attitude in connexion with methods for artificially increasing yield of latex. The problem to be solved is whether latex is a secondary waste product, the withdrawal of which from the laticiferous cells does not influence the vital processes, or whether it is a primary product, the withdrawal of which means increased activity on the part of the tree, in order to immediately replace that taken away. If the latter, then artificial methods of increasing yield beyond certain limits prejudices the health of the tree, while if it is waste material, any method of increasing yield might be employed, if the method of extraction, i. e. bark removal, &c., did not interfere with the normal processes. In estate practice rapid removal of bark, which implies insufficient time for a good renewal, is the limiting factor in the tapping process, not the quantity of latex extracted. The problem stated above is of prime importance for the future of the rubber industry. The investigation of physiological problems has been neglected in the past, partly because of the difficulties which surround investigations of this description in tropical countries, and partly because more obvious matters demanded immediate attention. It is to be hoped that the line suggested will receive earlier attention, though many years must elapse before results of practical value are obtained.



# A Systematic Analytical Study of certain North American Convallariaceae, considered in regard to their Origin through Discontinuous Variation.<sup>1</sup>

BY

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THE following is a précis of the main points in the original paper, the full publication of which is reserved until after the War :

The work is a systematic treatment of the Convallariaceae of North America which aims primarily to consider their relationships and phylogeny from the point of view of the mutationist. By analysis of the characters of the various species and genera, it is shown that many of the specific and generic differences are such as may have arisen through marked discontinuous changes. Indeed, many of the differences cannot be reasonably interpreted as the result of the gradual accumulation of smaller differences —the older Darwinian view of evolution. The paper is, therefore, an application to systematic work of the mutationist conceptions gained from experimentation. Its aim is to show how specific differences can be analytically treated in terms of definite and marked variations. It appears that apparently great external changes may be the result of a single germinal alteration, and that related species have often not been gradually differentiated, but have arisen by definite steps and probably in many cases without any aid from Natural Selection.

Many difficulties naturally arise in connexion with this or any other method of explanation, and a number of these difficulties are discussed in the paper.

Since the North American *Polygonatum*s have been very much confused and are not yet well understood, an account of this genus is published elsewhere.<sup>2</sup> The genus *Trillium* and its relatives, and the extensive literature on the variations of *Trillium*, are also treated in another publication.<sup>3</sup>

<sup>1</sup> A synopsis of this paper was presented before the Royal Society of Canada, at Ottawa, in May, 1917, by Dr. A. H. Mackay, Superintendent of Education for Nova Scotia.

<sup>2</sup> Gates, R. R. : A Revision of the Genus *Polygonatum* in North America. Bull. Torr. Bot. Club, vol. xliv, 1917, pp. 117–26, Pls. IV–VI.

<sup>3</sup> A Systematic Study of the North American Genus *Trillium*, its Variability, and its Relation to *Paris* and *Medeola*. Annals, Missouri Bot. Garden, vol. iv, pp. 43–92, Pls. VI–VIII, Map.

The term variety has been used in many senses, and it has therefore seemed desirable to draw a distinction between two classes of varieties, although it is not always possible to distinguish clearly between them. The sharply defined variety or *varietas abrupta* (written *var. abr.*) is the term used to designate those forms which differ from the type in an abrupt and clearly defined manner, suggesting that they have arisen from the type through a mutation and exhibit a unit-character difference. On the other hand, varieties which exhibit a series of intermediate stages are known as transitional varieties (*varietas transitionis*, written *var. trans.*). Although it is not possible to classify all varieties in this way, yet it is deemed useful to make this distinction in certain cases.

The original paper contains a mass of detail, so that only a few selected cases illustrating the point of view can be considered here.

The genus *Clintonia* contains six species—two in eastern and two in western North America, one in Japan and eastern Siberia, and one in the Himalayas. They are all well-marked species. The differences between *C. borealis* and *C. umbellulata* in eastern Canada and the United States have been considered elsewhere.<sup>1</sup> These two species are the most closely related, differing chiefly in size and number of flowers and the pubescence of the pedicels. There has been relatively little extinction between them, and the steps by which they have diverged have been relatively small. Of the two western species, *C. uniflora* was probably developed from the ancestors of *C. borealis* through a few steps, chiefly retrogressive, in the production of a single white flower.

The other western species, *C. Andrewsiana*, must have attained its present condition through several well-marked progressive steps: (1) great increase in size, (2) increase in number of flowers, (3) change in flower-colour to rose. All these changes could not have resulted from a single mutation, and hence a considerable amount of extinction must have taken place. A difficulty arises here, for while a stouter mutation might be supposed to triumph over a less robust original condition, yet there is no obvious reason why rose-coloured flowers should replace yellow ones. Breeding experiments and a cytological examination of the four species would help to answer such questions as these.

In *forma lateralis*, Peck, of the eastern *C. borealis* there are one or two lateral umbels on the scape, in addition to the terminal one. It is significant that this tendency in *C. borealis* is fully expressed in the constantly numerous lateral flower fascicles of *C. Andrewsiana*.

In *Smilacina* the two eastern species have corresponding western varieties, respectively *S. racemosa* var. *trans. amplexicanus* and *S. stellata* var. *trans. sessilifolia*. Although in the east these two species are relatively fixed and uniform, yet their western varieties show all intergrades to the

<sup>1</sup> On Pairs of Species. Bot. Gaz., vol. lxi, 1916, p. 181, Figs. 2, 3.

typical eastern forms, indicating that an evolutionary process of some continuous kind is in progress.

The variety *S. stellata* var. *mollis* (Farwell) comb. nov. is said to differ chiefly 'in having the leaves densely and permanently velvety pubescent all over the lower surface'. The specimens cited are from Michigan and the Black Hills of South Dakota. This pubescent variation is apparently parallel to *Maianthemum Canadense* var. *interius*, Fernald, the type of which came also from the arid Black Hills region and which differs from the species in being densely hirsute on the stems and the lower surface of the leaves. This case of a probable mutation which enabled the species to extend its range has been referred to elsewhere.<sup>1</sup>

There are eight recognized species of *Disporum* in North America, the remaining species being European or Asiatic. The distribution of the American species is roughly as follows: *D. lanuginosum*, from Ontario to Tennessee; *D. maculatum*, a restricted area in the mountains of Tennessee and North Carolina; *D. trachycarpum*, from the Canadian prairies south to Colorado and west to Oregon; *D. Smithii*, in the coast ranges from British Columbia to northern California; *D. oreganum*, distribution similar to that of *D. Smithii*, but extending farther inland; *D. trachyandrum*, in the Sierra Nevadas from Oregon to middle California; *D. Hookeri*, in a restricted area of the coast range of middle California; *D. parvifolium*, rare, in the Siskiyou Mountains of northern California.

These species are for the most part rather sharply marked. Nearly all of them are clearly distinguished from another species by one striking difference, accompanied in some cases by other minor differences. The nature of these differences is such as experience teaches us might have arisen through a single mutation. Furthermore, we are familiar in the Oenotheras with mutations in which one character has been markedly modified while others are only slightly altered. Thus *D. maculatum* differs from *D. lanuginosum* chiefly in having somewhat larger flowers, but most strikingly in having the perianth segments yellowish white with fine purple spots, instead of greenish.

Again, *D. trachycarpum* is sharply distinguished from *D. lanuginosum* and all other species in having beautifully reticulated fruits, whereas they are glabrous in nearly all other species. *D. trachycarpum* was considered a variety of *D. lanuginosum* by both Baker and Hooker, and it is by no means unreasonable to suppose that a single mutation may have produced it, though it differs from *D. lanuginosum* also in having fewer seeds, and stamens as long as the perianth.

*D. oreganum* differs from the eastern species in having an entire instead of a three-cleft stigma, and from its nearest relative, *D. trachycarpum*, in its ovate (not globose) pubescent or glabrous but smooth (not reticulated)

<sup>1</sup> The Mutation Theory and the Species-concept. Amer. Naturalist, vol. li, 1917, p. 583.

fruits ; also in certain minor characters of foliage and pubescence. But the two species run into each other and probably intercross.

*D. Smithii* and *D. Hookeri* form a pair differing in having respectively, (1) larger whitish and smaller greenish flowers, (2), densely short-hairy or glabrous style and ovary, (3) leaves mostly subcordate or cordate at base ; (4) in *D. Smithii* the leaf margins are ciliate. *D. Hookeri* differs from all the others in having flowers which are broad and truncate at base. *D. trachyandrum* probably originated from *D. Hookeri* through a mutation. It differs strikingly in having hairy anthers, but the leaves are also less deeply cordate and distinctly broader towards the apex, and the ovary is glabrous.

Thus it will be seen that for the most part the species of this genus are sharply separated from each other by 'presence and absence' characters, and only to a minor extent by quantitative characters. The latter are likely to be often merely by-products, as it were, of the more striking germinal changes. It is impossible to suppose that, e.g., stages in the development of the reticulation of the fruit, the lobing of the stigma, or the hairiness of the anthers could be of use to the plant. Indeed, it seems fairly clear that such differences are the result of sudden 'chance variations' and have no significance in the economy of the plant's life. Having appeared, heredity perpetuates them, and so new varieties or species come to be established. Their comparative recency of origin can perhaps be judged to some extent by the relative areas they now occupy.<sup>1</sup> We should then expect *D. maculatum* and *D. trachyandrum* to have originated relatively recently, while *D. trachycarpum*, which occupies a much wider area, may have originated from *D. lanuginosum* at an earlier time. There are, however, difficulties in applying this view in detail even to such a relatively simple genus as *Disporum*. But the underlying idea of definite variations arising in certain localities and afterwards spreading, rather than the hypothetical accumulation of small differences and the subsequent elimination of intermediates, corresponds with the facts of such a genus as we observe them. In the light of the facts of mutation, single definite steps, or in some cases a few steps, from species to species are by no means improbable, and it appears that all the conditions in the North American *Disporums* can be explained by their aid, without resorting to the accumulation hypothesis.

The genus *Uvularia* is confined to eastern North America. It contains two species, *U. perfoliata* and *U. grandiflora*, and a third, *U. flava*, which is known only from a figure and description in Smith's 'Exotic Botany', and is probably a hybrid between the other two. Both these species range from Quebec nearly to Florida, but *U. grandiflora* extends farther west into

<sup>1</sup> This was originally written before the interesting papers of Willis appeared, applying this conception to the floras of Ceylon and New Zealand.

See Willis, J. C. : The Evolution of Species in Ceylon, with reference to the Dying Out of Species, Annals of Botany, vol. xxx, 1916; The Distribution of Species in New Zealand, ibid., pp. 437-57; and several other recent papers.

Kansas and Minnesota. The striking distinctions are in the presence or absence of papillae on the perianth segments, and in the ventral surface of the leaves being glaucous or pubescent. *U. grandiflora* may possibly be a cell-giant of *U. perfoliata* which has since undergone two or three mutations, such as (1) loss of papillae from the perianth segments and (2) acquirey of pubescence instead of glaucousness.

The genus *Oakesia* was formerly included in *Uvularia*, to which it is closely related. It includes four species occupying much the same area as *Uvularia*. The common ancestor of the two genera probably had the sessile leaves and ovoid fruits of *Disporum*. One or more mutations produced from this stock the perfoliate leaves of *Uvularia*, while another series of mutations led to the peculiar winged capsules, angular stem, and other features of *Oakesia*. The great similarity of the two genera in habit and flowers shows that there has been little extinction between them.

The only other genus to which reference will be made here is *Streptopus*, in which three American species are recognized. *S. amplexifolius* and *S. roseus* make an excellent pair of species, as pointed out elsewhere.<sup>1</sup> *S. longipes*, Fernald, described in 1906, is at present known only from Michigan, New Hampshire, and Campobello Island, New Brunswick. It differs from *S. roseus* chiefly in having trigonous instead of subglobose berries. The flowers may also be paler and the root-stocks longer and more slender, but these are fluctuating and inconstant characters. This form has probably originated from *S. roseus* through a mutation, and furnishes a very good instance of a germinal change in which the main difference has arisen in one organ, with minor, more or less fluctuating differences in other parts of the plant.

Most of the specific and generic characters considered in this paper as differentiating the members of the Convallariaceae are such as are unlikely to be of any advantage to the plant. They probably appeared as germinal variations, and have since been perpetuated and have enlarged in various directions their area of occupation.

<sup>1</sup> On Pairs of Species. Bot. Gaz., vol. lxi, 1916, p. 185, Figs. 4, 5.



# Organic Plant Poisons.

## II. Phenols.

BY

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With eighteen Figures in the Text.

THE phenols form a group of organic compounds that suggest possibilities with regard to the partial sterilization of soils. On this account experiments have been carried out to gain some idea of the action of these substances on the plant itself, apart from the partial sterilization effects which occur in treated soils. In soil culture the action is largely, if not chiefly, indirect, by way of the increase of the available food supply; this indirect action is usually so strongly marked that any direct action, whether beneficial or adverse, is masked. In water cultures the partial sterilization effect is absent on account of the lack of available organisms in the shape of protozoa and bacteria, so that the direct action of the phenol on the plant may be observed.

The whole series of experiments were carried out systematically on parallel lines, to admit of comparisons between the action of one phenol and another. The solutions in each case were made up on the basis of the molecular weights of the poisons, the strongest solution in each case containing M/100 grammes per litre, each of the succeeding strengths being one-fifth of that preceding—i.e. M/100  $\times \frac{1}{5}$ , M/100  $\times 1/5^2$ , . . . M/100  $\times 1/5^6$ —all being matched against controls receiving no poison. In most cases the usual Rothamsted solution was used, but occasionally comparisons were made with results from a weak nutrient solution.

*Strong nutrients (Rothamsted solution).      Weak nutrients.*

		grm.	grm.
Potassium nitrate . . . . .	.	1.0	0.2
Sodium nitrate . . . . .	.	—	0.5
Magnesium sulphate . . . . .	.	0.5	0.1
Calcium sulphate . . . . .	.	0.5	0.1
Potassium di-hydrogen phosphate . . . . .	.	0.5	0.1
Sodium chloride . . . . .	.	0.5	0.1
Ferric chloride . . . . .	.	0.04	0.04
Distilled water, to make up . . . . .	.	1 litre	1 litre

### *Phenol.*

Parallel sets of peas were grown with strong and weak nutrients (Sept. 24 to Dec. 5). M/100 proved to be a virulent poison. Within two days of insertion the parts of the roots above the solutions were all utterly shrivelled, whereas within the solution they were very white, rather swollen, and flabby, with a most unnatural appearance. The

shoots seemed to live on for some time longer, though probably this was merely an external appearance of vitality. They remained green for a fortnight in the weak nutrients and for nearly three weeks in the strong. The phenol exerted an antiseptic action for some long time, in spite of the death of the roots, but at last moulds began to form on the surface of the solutions.

With a lower concentration,  $M/100 \times \frac{1}{5}$ , the difference in the action of the poison in strong and weak nutrients was evident from the first. With the strong nutrients root growth was apparently quite normal for the first few days, after which it was very much hindered, though no constriction

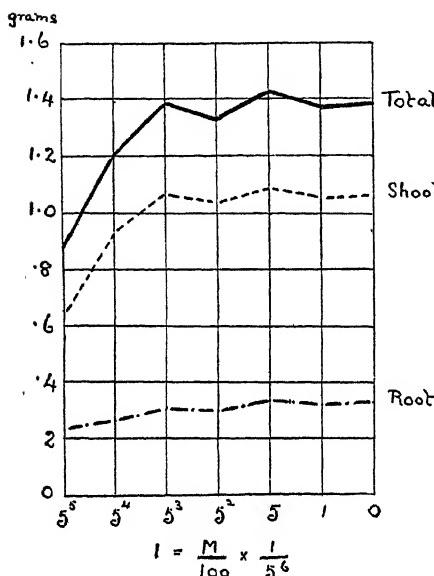


FIG. 1. Average dry weights of ten series of pea plants grown in strong nutrient solutions in the presence of differing amounts of phenol. Sept. 24-Dec. 5, 1914.

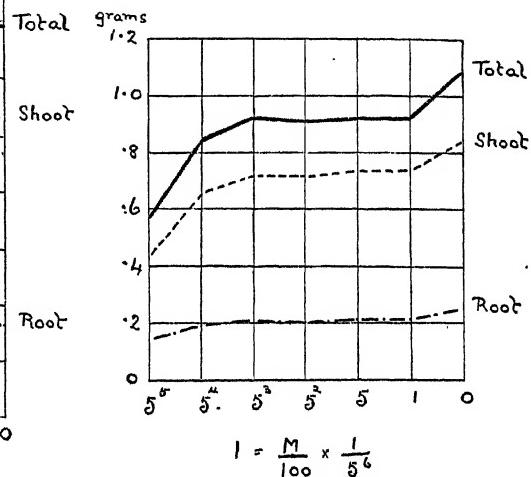


FIG. 2. Average dry weights of ten series of pea plants grown in weak nutrient solutions in the presence of differing amounts of phenol. Sept. 24-Dec. 5, 1914.

or unnatural coloration of roots was manifest. With weak nutrients, on the contrary, the roots showed an inclination to be constricted and pink in colour near the tips within two days, the tips themselves being dead white and somewhat swollen. Later on, in both sets the shoots became very weak and the roots bunched owing to the belated formation above water of laterals which showed great reluctance to enter the solutions. After about six weeks the roots began to elongate, but the start into activity occurred some days earlier in the strong nutrients, the ultimate improvement in the type of root being much more marked.

With the next concentration,  $M/100 \times 1/5^2$ , all the plants started off in a similar way, with shoots much like those of the control plants and roots of a normal type; gradually there was a falling off in the rate of growth. After about three weeks the difference between the sets was more manifest.

The plants in strong nutrients continued to grow in a normal way, forming laterals within the solutions. Those in weak solutions, on the other hand, again formed numerous laterals bunched together above the surface of the solutions, which did not proceed to elongate and enter the water for several days or a fortnight. It is very noteworthy that, in spite of the striking difference in the development of the roots, the ultimate dry weights in each case did not fall very much below the weights of the respective controls, though far greater differences were shown by the shoots.

With all concentrations below the last described the growth of the plants compared very favourably with that of the controls all the way through ; there was no abnormality in development or colour, and the dry weights proved to be fairly uniform, giving no signs of stimulation.

A comparison of the dry-weight curves shows that with every concentration, from the least to the greatest, the growth of the plants in strong nutrients was uniformly better than in weak, the curves running fairly parallel. The earlier recovery of the roots in strong nutrients is an instance of the masking action exercised by food salts on the action of a toxic substance, as has been seen with inorganic poisons.<sup>1</sup> It is probable that the phenol is gradually decomposed by oxidation, thereby getting less in quantity, and that the extra energy generated by the more liberal food supply in strong nutrients enabled the roots to overcome the inhibiting action before as much decomposition had occurred as was necessary with weak nutrients.

In another series carried out later in the year (Oct. 15 to Jan. 30) with strong nutrients the toxicity of the higher concentrations of phenol was rather greater than in the previous experiment. This was shown by the abnormal and delayed root growth being developed with less poison, and was probably due to the fact that the vitality of the plants is lower in the depth of winter, so that they had not so much energy to withstand the poison. When once a fair start was made, however, the ultimate recovery was quite good.

An initial series of barley with phenol was grown early in the year—March 5 to April 17. M/100 proved instantly fatal. The day after the plants were started the roots were very translucent and rapidly became flaccid. The shoots kept their green colour for a time without making the least growth, but after ten days they turned an unhealthy yellow brown colour and rapidly died off. M/100  $\times \frac{1}{5}$  permitted a little shoot development, but no root growth took place for a month, and the roots were brownish. By this time the shoots were very small, variable, with the base of the stems deep purple-red in colour—a sure sign of starvation, due in this case to insufficient nutriment being taken by the roots in the midst of plenty. At length the roots began to grow a little ; they became bunchy above the solutions without entering, and they maintained this attitude to the end of the experiment, refusing to put out long laterals into the solutions. The

<sup>1</sup> Brenchley, W. E. : Inorganic Plant Poisons, 1914, p. 20.

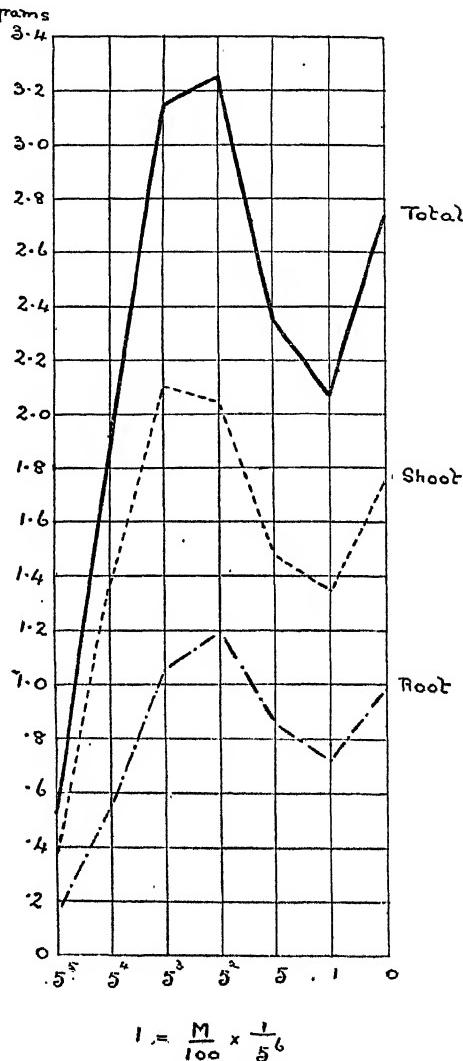
start into activity of root growth was sharply reflected by the shoots, which improved greatly and lost some of their purple colour, though they remained small to the end.

With the next strength,  $M/100 \times 1/5^2$ , a considerable initial check was evident, the roots being pinkish in colour. In about a fortnight the roots reached the bunched stage, and after that rapidly improved, sending out long laterals into the solutions and eventually becoming almost normal in type. The effect of the initial check was visible in the brown discolouration which remained in the older part of the root, and also in the depressed dry weight.

Growth steadily improved as the concentration decreased. There is just a hint of a possible stimulation of growth with  $M/100 \times 1/5^3$ , as the plants are very considerably above the controls in weight. A similar result was obtained with another series later in the year; but there is not sufficient evidence to justify more than a suggestion that some stimulation may possibly occur at this point, *provided the nutrient solutions remain unchanged throughout the course of the experiment*. The rise does not occur if the solutions, and with them the supply of phenol, are renewed.

In order to test the hypothesis that the decomposition or oxidation of the poison affects the plant recovery in the higher concentrations, three parallel sets of barley were grown:

- (1) Solutions never changed (Fig. 3).
- (2) Solutions changed once during the experiment (Fig. 4).
- (3) Solutions changed twice during the experiment (Fig. 5).



$$I = \frac{M}{100} \times \frac{1}{5^6}$$

FIG. 3. Average dry weights of ten series of barley plants grown in strong nutrient solutions in the presence of differing amounts of phenol. Solutions never changed. April 27–June 15, 1915.

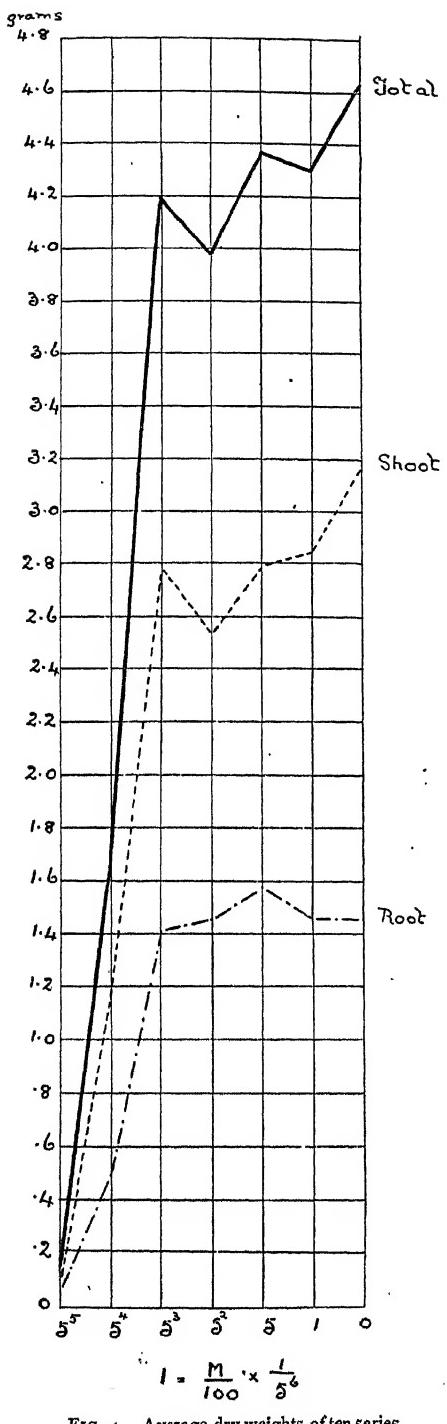


FIG. 4. Average dry weights of ten series of barley plants grown in strong nutrient solutions in the presence of differing amounts of phenol. Solutions changed every three weeks.

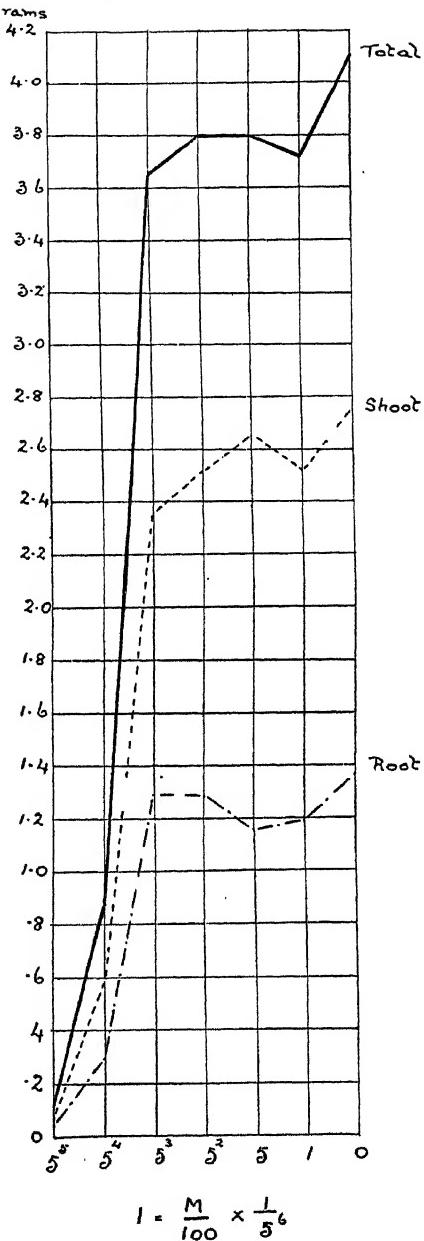


FIG. 5. Average dry weights of ten series of barley plants grown in strong nutrient solutions in the presence of differing amounts of phenol. Solutions changed every two weeks. April 27-June 15, 1915.

With the controls and with the lower strengths in which the phenol had apparently reached its indifferent point the growth of the barley was much better when the solutions were renewed than when they were never changed. This is in accordance with other work and is independent of the concentration of the nutrient solution.<sup>1</sup> With the higher strengths of phenol, which are toxic, increase of growth did not run parallel with renewal of food solution; but, on the contrary, the actual growth as shown by the dry weight was in inverse ratio to the frequency of renewal.

TABLE I.  
*Dry Weights of Barley Plants. Mean of ten Plants.*

	Sols. never changed. grm.	Sols. changed once. grm.	Sols. changed twice. grm.
M/100 × 1/5	0.525	0.150	0.117
M/100 × 1/5 <sup>2</sup>	1.910	1.651	0.871

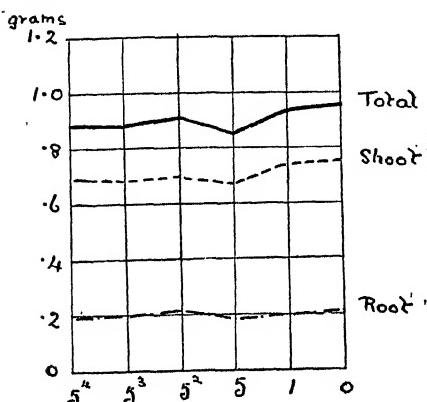
The figures in Table I show very clearly that root recovery and the ultimate improvement of growth are associated with the decomposition of the poison. With M/100 × 1/5 the decomposition had not proceeded far enough in any case to allow the inhibitory toxic factor to be more than counterbalanced by the inherent vitality of the root. Consequently root recovery could not begin to set in before a fresh supply of phenol was presented to the plant, even when the solution was only changed once. When no renewal was made the roots were able to make some amount of recovery, but the strength of the poison was so great that this was comparatively slight.

With M/100 × 1/5<sup>2</sup>, on the other hand, the decomposition of the poison was counterbalanced at an earlier date by the plant's vitality, so that root recovery set in before the renewal, when the solutions were only changed once. When growth had made a fair start the plants were more resistant to the action of the fresh supply of phenol, so that the ultimate growth was not so very far behind that of the plants with which no renewal had been made. When the solutions were changed more frequently, root recovery was still in abeyance or had only just begun when a fresh supply of poison was presented, consequently recovery was thrown back considerably, and though the roots proceeded slowly to form new laterals a further check took place with the second renewal, and the ultimate growth was much less than that of all the other plants, in which the solutions were changed once or not at all. By the time of cutting the plants had apparently got a grip on life, and judging by their appearance it is possible that if they could have been grown on longer they might have made up their deficiency at a more rapid rate. It seems that when once growth is fairly under way and vigorous, the plants can cope with a strength of poison which is most deleterious at an earlier stage of development.

<sup>1</sup> Brenchley, W. E.: Ann. of Bot., vol. xxx, pp. 77-90.

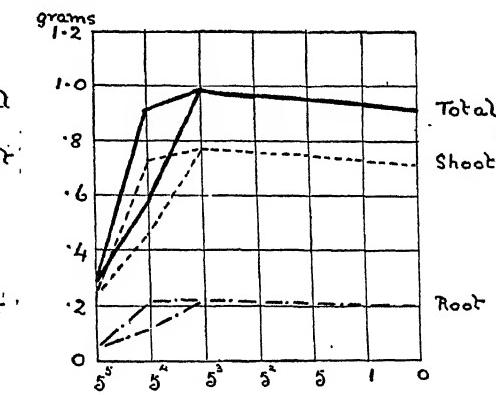
## Cresol.

Very little seems to be known about the occurrence of the cresols in the Vegetable Kingdom, nor of their effect on plant life. Wehmer<sup>1</sup> states that the flowers of *Acacia Farnesiana* yield *p*-cresol, and that certain members of the family Burseraceae contain *m*-cresol. The three cresols are



$$I = \frac{M}{100} \times \frac{1}{5^6}$$

FIG. 6. Average dry weights of ten series of pea plants grown in strong nutrient solutions in the presence of differing amounts of *o*-cresol. Oct. 5-Dec. 21, 1914.



$$I = \frac{M}{100} \times \frac{1}{5^6}$$

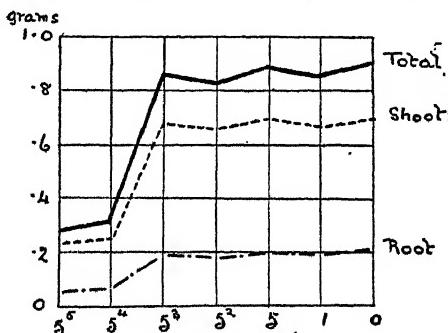
FIG. 7. Average dry weights of ten series of pea plants grown in strong nutrient solutions in the presence of differing amounts of *m*-cresol. Oct. 6-Dec. 21, 1914.

N.B. 5<sup>4</sup>. Upper curve = mean of plants which made good recovery. Lower curve = mean of plants which made poor recovery.

all toxic, but in varying degree. Tollens<sup>2</sup> indicates that in animal investigations *o*-cresol is about as toxic as phenol, *p*-cresol being more poisonous, *m*-cresol rather less so. No reference to this relative toxicity with regard to plants has so far come to light. Consequently barley and peas have been tested with all three forms in the usual way.

#### Peas (Figs. 6, 7, 8).

All the plants were started at the same time, and the growth of the series treated with the three cresols was remarkably



$$I = \frac{M}{100} \times \frac{1}{5^6}$$

FIG. 8. Average dry weights of ten series of pea plants grown in strong nutrient solutions in the presence of differing amounts of *p*-cresol. Oct. 5-Dec. 21, 1914.

<sup>1</sup> Wehmer: Die Pflanzenstoffe. 1911.

<sup>2</sup> Tollens, quoted by Kober, R.: Lehrbuch der Intoxikationen. 2. Auflage. 1902-6.

uniform, so that comparison was much facilitated. In every case the strongest poison, M/100, was almost immediately fatal, as within two days the roots were quite flabby and most unnaturally white, no attempt at lateral formation having been made. Within a week the shoots had died off in a way that had never been witnessed before in the greenhouse with any other poison, whether organic or inorganic. The stem shrivelled from the seed to the leaves, the whole plant was prostrate, the leaves limp and wilting, reminding one irresistibly of stale greens in a neglected corner of a greengrocer's shop. The strongly antiseptic action of the cresol was shown by the fact that, in spite of the early death of the plants, no sign of mould growth appeared either on the plants or on the solutions during the six weeks that the cultures were retained in the house.

With the next concentration,  $M/100 \times \frac{1}{5}$ , all the plants started alike, but differences manifested themselves a little later on. Within two days the tip of the root became constricted and white, and, except in the case of *o*-cresol, somewhat flabby.

With *o*-cresol practically no growth was made, but for some time the plants continued to look healthy, as though their development had been merely arrested, not permanently inhibited. After about a month the roots became flabby, without forming any laterals. Within another fortnight the leaves began to wilt, the roots shrivelled just below the seeds so that the plants were unsteadily prostrate, and mould put in an appearance. Evidently the *o*-cresol was not strong enough to prevent the latter phenomenon from occurring, as with the previous concentration. By the end of the experiment every plant was dead and shrivelled.

With *m*-cresol the shoots made a considerable amount of growth, so that for the first month they were much better than those in *o*-cresol. Nevertheless symptoms of degeneration set in at an earlier date, as by the end of the month many of the leaves were flaccid, the plants were prostrate, and mould formation had begun. In spite of this the plants clung to life more tenaciously than with the *o*-cresol, and at the end of the experiment there were three survivors, with rather dark little roots and very small pale shoots, which had endeavoured to flower.

*p*-Cresol at this concentration seemed, at one and the same time, to be more toxic than either of the others and yet to allow of greater resistance or recovery. A larger part of the root became flabby at an early date, the shoots were less well developed than any others, and a strong growth of mould appeared on roots and solutions within a fortnight of the start. Most of the plants died fairly soon, the shoots becoming flaccid and shrivelled, the seeds shrivelling also. The development of mould was very strong, far more so than with either of the other cresols. Four plants survived out of ten; these possessed small shoots which had tried to flower. The roots were very poor, brown or black in colour, but they had tried to put out a few short, whitish laterals. Thus, though most of the plants were

killed earlier than with *o*- or *m*-cresol, the survivors with this strength of *p*-cresol were the only plants that had formed even apologies for laterals in the presence of this amount of poison.

The greater toxicity of *p*-cresol was far more evident with the next concentration,  $M/100 \times 1/5^2$ . The plants started off all right, but very soon both shoots and roots began to fall behind in development. The laterals were at first short, curled, and few in number, and the roots remained poor to the end, and were also hampered by a strong growth of mould. The shoots were variable, some inclined to be flaccid, and all of a sickly green colour. The dry weights of these plants were far below those with the other cresols, as is shown in Fig. 8. With both *o*- and *m*-cresol the ultimate growth of most of the plants was very similar, but the course of development was rather different. With *o*-cresol growth was like that of the controls for about a fortnight, and the shoots continued to keep pace all the way through. The roots, however, became very yellow, bunchy, short, and not at all like the controls in type. This phase lasted for about a fortnight, and then the laterals began to elongate once more. The roots remained somewhat abnormal, being brownish and short at the time of cutting. With *m*-cresol again, the shoots were much like those of the controls all the way through. The roots formed plenty of laterals, but almost from the beginning they were rather short, thin, and of an unhealthy yellowish colour. The roots soon became bunchy and the colour deepened. Eventually the laterals elongated and the roots improved rapidly, approaching the normal more closely; but the final development of the plants was rather variable, some making a good recovery, comparing closely with the controls, others remaining weaker though much stronger than those in *p*-cresol.

With the next concentration,  $M/100 \times 1/5^3$ , the differences in the action of the various poisons had almost disappeared. The plants with *o*-cresol were similar to the controls from beginning to end, without any abnormality in development. With *m*- and *p*-cresol some trace of poisoning was still evident; with the former some of the laterals tended to be rather thin and of an unusual type, with numerous thin side shoots; while with the latter some of the roots had flabby tips at the first, though lateral formation was normal. All plants pulled up well later on, and the dry weights in all three sets were very similar. Below this strength the action of the poison could not be discerned in any case, all plants comparing well with controls.

On the whole, it may be said that *p*-cresol is more toxic in high concentration than *o*- or *m*-cresol, and that probably *m*-cresol is slightly more poisonous than *o*-cresol, though the difference is not well marked. The apparent anomaly exists, that in spite of this absolute range of toxicity the highest concentration of *o*-cresol (the *least* poisonous) kills all the plants outright, whereas the same strength of *p*-cresol (the *most* poisonous) allows the plants to make a little growth, and also to attempt to put forth laterals, which was not the case with *o*- or *m*-cresol.

Barley (Figs. 9, 10, 11).

M/100. With *o*- and *p*-cresol root growth was checked almost as soon as the plants were put into the solutions, but with *m*-cresol a little root development was made for a few days before growth was inhibited. All roots were very white, and no mould appeared in any case. The shoots did not develop at all, but the single leaf turned a brownish yellow colour and expanded to its fullest extent, contrasting strongly with the usual habit of barley shoots, which when poisoned strongly remain rolled up at death.

M/100  $\times \frac{1}{5}$  was very toxic in every case. The shoots were rather less

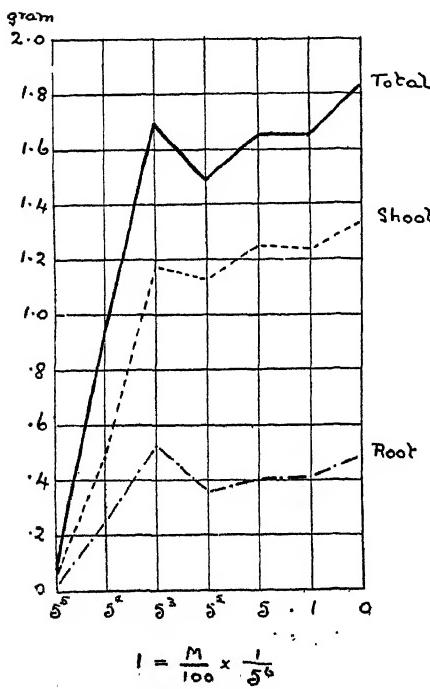


FIG. 9. Average dry weights of ten series of barley plants grown in strong nutrients in the presence of differing amounts of *o*-cresol. March 8—April 21, 1915.

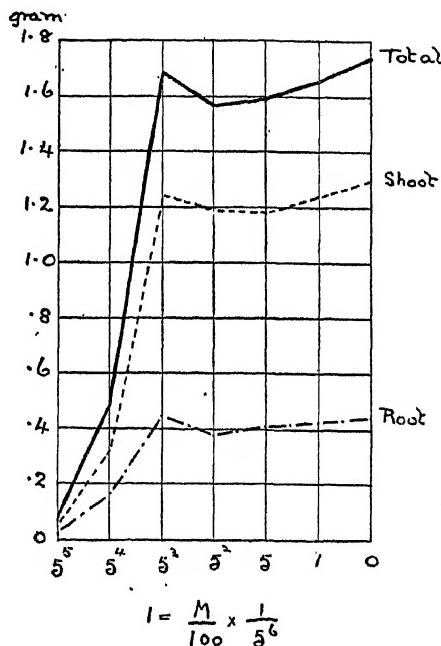


FIG. 10. Average dry weights of ten series of barley plants grown in strong nutrients in the presence of differing amounts of *m*-cresol. March 11—April 24, 1915.

checked, and formed two little leaves, but development did not go beyond this. The familiar red coloration due to starvation appeared at the base of the stem. By the end of the experiment the plants were all but dead, the lowest leaves being deep yellow and the root dying. Mould appeared on the roots, being most strongly marked with *p*-cresol.

M/100  $\times \frac{1}{5^2}$ . The greatest differentiation in the action of the cresols occurred with this concentration. With *o*-cresol the roots were some time before they started into growth; when they did begin to develop, stubby laterals were formed. Eventually these began to push down into the solutions, but they were of a thick type, unlike normal barley roots. The

ultimate results were variable, some plants being strong and healthy, others small and weakly, but the general growth was much better than in either of the other cresols. With both *m*- and *p*-cresol root growth was greatly checked at first; the roots became bunched, not entering the solution for some time, but later on thick laterals were formed, which pushed down into the water. The shoots were small and fairly healthy, but they showed purple coloration in the stem. The recovery was less in both cases than with *o*-cresol.

$M/100 \times 1/5^3$ . With *o*- and *m*-cresol growth was normal from the first, though for a time some slight check to the rate of growth was evident. This was overcome later on, and the plants pulled up to the level of the others receiving less poison. With *p*-cresol the roots were very short and bunched at the beginning, and did not begin to struggle ahead for about a fortnight. When once the long laterals began to form and enter the solutions the improvement in growth was very rapid and the plants made up most of their leeway.

It is noticeable that in all three cases the barley receiving the next strength of poison,  $M/100 \times 1/5^4$ , made less growth than with the higher concentration just described. This drop was chiefly in the root, being much less marked in the shoot. As the concentration decreased from this point, the dry weights increased steadily towards that of the control. As this happened consistently with all three cresols it is quite probable that it is a true phenomenon. With the  $M/100 \times 1/5^3$  strength the balance of warfare between the plant and the poison is very nicely balanced, and it is possible that in its strong efforts to gain the upper hand the plant developed a very extended root system and made a fuller use of the food supply, resulting in a greater dry weight. Apparently the whole effect of the poison is not lost with rather lower concentrations, but the toxic action is so slight, that the plant does not make the same effort to overcome it by

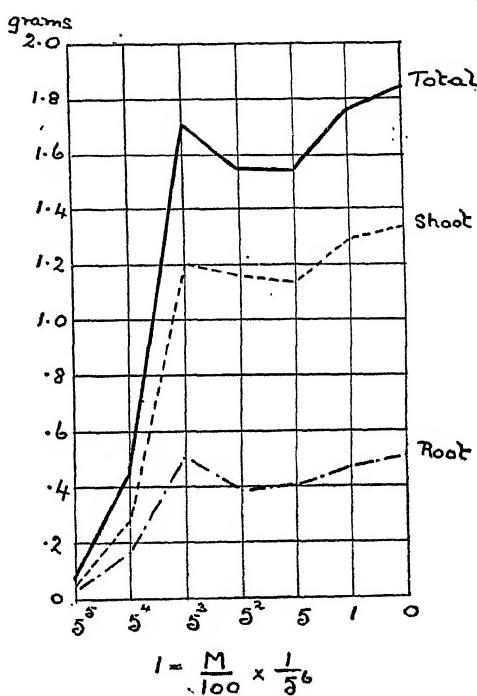


FIG. 11. Average dry weights of ten series of barley plants grown in strong nutrient solutions in the presence of differing amounts of *p*-cresol. March 13–April 26, 1915.

means of greater root growth, and consequently the dry weights fall behind somewhat. The increase observed in the former case may be an instance of a local stimulation whereby increased growth is made to enable the organ concerned to cope with some special circumstance affecting the plant economy. This is quite different from stimulation in the ordinary sense, whereby the rate of growth of the whole plant is affected, giving corresponding increase in the dry weights of both root and shoot.

In this connexion it is interesting to make a comparison of the shoot-root ratios in relation to the actual weights. With high concentrations of the poisons (except when root growth was so inhibited that death occurred early, so vitiating the value of any figures) the development of the roots in comparison with the shoots is much above normal, so that the shoot-root ratio is very low. This is what might be expected, as the action of the poison probably prevents a sufficient supply of food from entering the plant, so that if the latter is able to make any growth at all it concentrates its efforts first on extending the root system as far as possible in order to enlarge the absorbing area, improvement of the shoot following later, if at all, i. e. if the increase of root area is really associated with increased absorption. In the case under consideration with a particular concentration of poison,  $M/100 \times 1/5^3$ , the shoot reached a normal size, but to enable this amount of growth to be made the roots had to develop to an abnormal extent, so that the shoot-root ratio is still low. Below this concentration, when the action of the poison is very weak and the resistance of the plant is almost nil, the ratio approximates closely to the normal.

#### *Shoot-Root Ratio.*

	<i>Ortho-cresol.</i>	<i>Meta-cresol.</i>	<i>Para-cresol.</i>
Control	2.744	2.974	2.638
$M/100 \times 1/5$	5.700	1.960	1.200
$M/100 \times 1/5^2$	1.955	1.988	1.681
$M/100 \times 1/5^3$	2.252	2.776	2.367
$M/100 \times 1/5^4$	3.156	3.133	3.013
$M/100 \times 1/5^5$	3.110	2.907	2.826
$M/100 \times 1/5^6$	2.983	2.939	2.748

#### *Resorcinol.*

The effect of very strong ( $M/100$ ) resorcinol on peas (Fig. 12) was rather remarkable, on account of the great difference in the initial action of the poison on the roots and shoots. The roots were killed at once, as within two days they were white and flabby, and the parts above the solutions had begun to shrivel. In spite of this the shoots started into growth and made very fair development for several days, so that they were nearly as good as the controls, although by the end of the first week the roots were infested with a strong growth of mould. After ten days the shoots were much stronger than any others that had received the same concentration of

other phenols, as cresol, orcinol, pyrogallol, &c., the amount of shoot growth being most surprising considering the state of the root. Perhaps the effect of the resorcinol was in some way to give a stimulus to shoot production, or at least to allow it to go on unhindered, so that although no fresh nutrient was being absorbed on account of the death of the root, yet the shoot was able to make use of the stores in the seed and develop for some time wholly at their expense. This may indicate that this particular poison is primarily local in its action, and that though it kills the root, the lethal

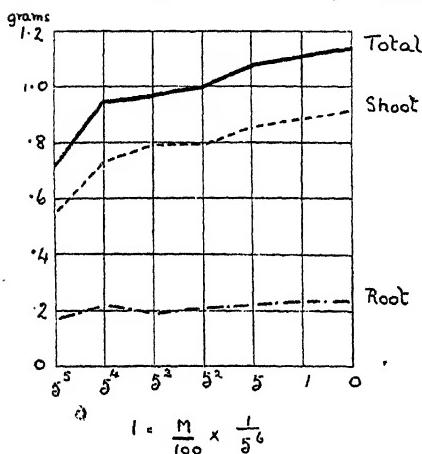


FIG. 12. Average dry weights of ten series of pea plants grown in strong nutrient solutions in the presence of differing amounts of resorcinol. Sept. 26-Dec. 17, 1914.

or paralysing influence is not transmitted to the shoot at once, but only makes itself felt after some time has passed.

With a rather lower concentration,  $M/100 \times \frac{1}{5}$ , the resorcinol did not make itself felt for several days, and even then the roots were much more affected than the shoots. The

roots gradually turned brown, then black; the laterals were weak and were usually formed above the solutions. At a still later date recovery set in, and long straight laterals were pushed into the solutions, so that at the end of the experiment most of the plants looked fairly healthy, and were not so very deficient in dry matter. Lower concentrations had little effect on

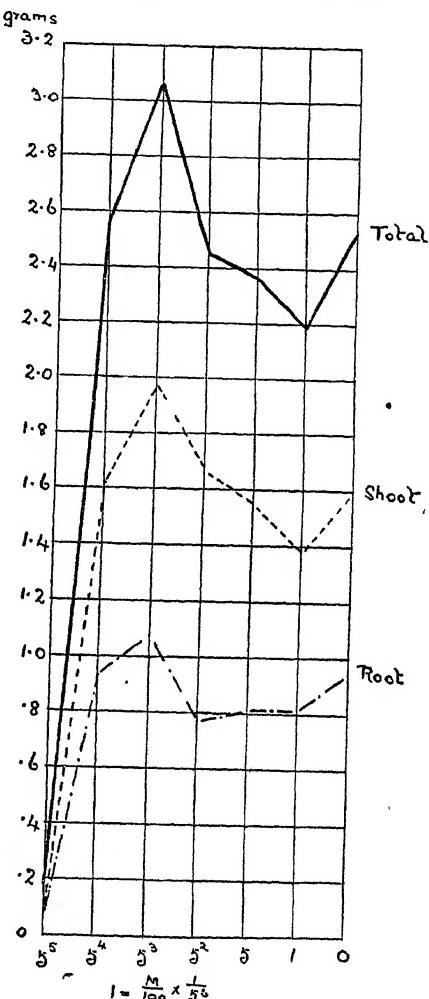


FIG. 13. Average dry weights of ten series of barley plants grown in strong nutrients in the presence of differing amounts of resorcinol. March 16-May 3, 1915.

the plants, except that in some cases the roots were rather discoloured. On the whole, to peas resorcinol is about as toxic as phenol itself, and much less toxic than most of the others of the phenol group.

Barley is, as usual, more sensitive than peas (Fig. 13). With strong concentrations, including  $M/100 \times \frac{1}{5}$ , the usual phenomena of intense poisoning were noticed—belated start into growth, bunching of roots owing to reluctance of laterals to enter the solutions, poor shoot development, with purple coloration. No recovery was noted in these cases, so the dry weight remained low. With rather lower concentrations, again as usual, an initial check was followed by recovery—recovery so complete that the dry weights were fully equal to controls. No stimulation nor toxic action was observed in the greater dilutions of poison.

### *Pyrocatechol.*

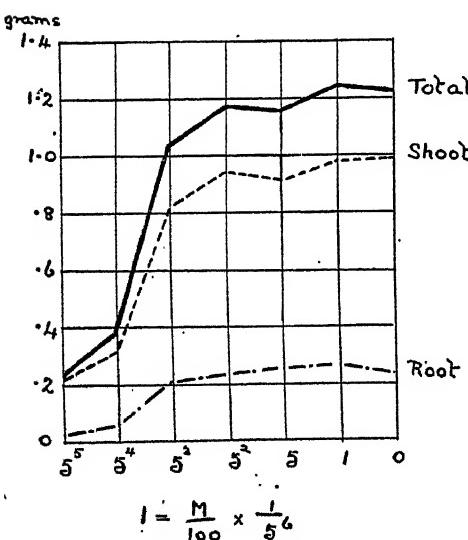
The general course of events was the same as with other phenols with which root recovery takes place at certain concentrations.

Pyrocatechol is more toxic than many of the others, as with both peas and barley the harmful effects were noticed much farther down the scale. The behaviour of pea plants with  $M/100 \times \frac{1}{5}$  poison was more noticeable (Fig. 14). The roots were killed beyond recovery at the very beginning and rapidly became grey, flabby, and mouldy. In spite of this the shoots continued to drag out an existence, and six weeks later actually came out into flower, though naturally the blooms were very small and poor. This is still more remarkable in that the concentration,

FIG. 14. Average dry weights of ten series of pea plants grown in strong nutrients in the presence of differing amounts of pyrocatechol. Sept. 29-Dec. 17, 1914.

$M/100 \times \frac{1}{5}$ , was not on the border-line of indifference, since with only one-fifth the strength,  $M/100 \times \frac{1}{5^2}$ , very little root recovery occurred in any case, and even when it did take place it was only to a slight degree.

The continuance of shoot growth after the death of the root was shown by the shoot-root ratio, which reached 10.5. As soon as root recovery set in the ratio rapidly fell towards that of the controls, which is about 4.2.



### Pyrogallol.

Owing to shortage of the supply it was only possible to test the effect of pyrogallol and phloroglucin upon peas. Pyrogallol was very poisonous, the toxic effect being noticeable with comparatively low concentrations (Fig. 15). High strengths killed the plants outright, and rather lower strengths allowed of well-marked root recovery. The poisoned roots became intensely black in colour, so that when recovery set in the contrast between the black root and the fresh white laterals was very marked. Decomposition of the solution took place very rapidly. The stronger solutions became very yellow and developed much heavy black or dark brown precipitate. The discolouration of the roots was still noticeable in the presence of so little pyrogallol that the solutions themselves were not turned but retained their usual colour. The mould development was rather unusual, as fungus was evident in the precipitate as well as floating on the surface of the solution. That among the precipitate included a chlamydospore condition of a *Nectria*, possibly *N. cinnabarina*, Fr., and a mycelium which was producing myriads of budding conidia. The surface mould was a starved condition of *Penicillium glaucum*, Link.<sup>1</sup>

### Phloroglucin.

This proved to be somewhat less toxic to peas than the pyrogallol (Fig. 16). No change occurred in the colour of the solution and the roots that were killed turned yellowish, without darkening much. After the moribund shoots lost their green colour they took on a curious reddish-brown colour in their stems, and the leaves turned brownish. Plants with  $M/100 \times \frac{1}{5}$  did not show the usual phenomena of arrested growth followed by later recovery. Instead the laterals were formed at the beginning, but made very poor growth above the solution, and very little in it. Later on, they grew out but were short, and became thick and yellow, and still later they became spindle-shaped, being stubby and thickened in the middle. The roots eventually blackened, and retained their abnormal character to the last, though they developed a few straight greyish-white laterals. With

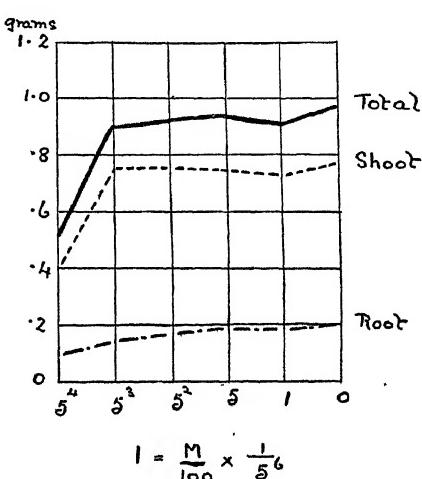


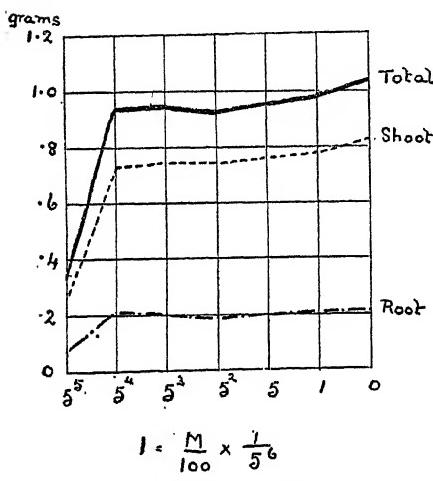
FIG. 15. Average dry weights of ten series of pea plants grown in strong nutrient solutions in the presence of differing amounts of pyrogallol. Oct. 9, 1914-Jan. 19, 1915.

<sup>1</sup> Moulds identified at Kew by the kindness of the Director.

a lower strength of poison the laterals showed an inclination to behave the same way at first, but before very long they pushed out well and the plants improved very greatly, approaching the controls. The shoot-root ratio remained fairly constant all through. The same mould as was obtained with pyrogallol (*Nectria cinnabarina*) was observed floating in the solution.

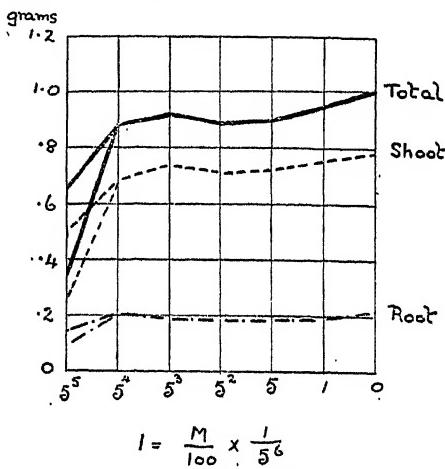
### Orcinol.

Strong solutions of orcinol, M/100, acted unfavourably on the roots of peas from the outset; as the tips became flabby within twenty-four hours of insertion in the solution, and within three days the whole root was involved. Within a week all the roots were flabby and constricted and



$$I = \frac{M}{100} \times \frac{1}{5^6}$$

FIG. 16. Average dry weights of ten series of pea plants grown in strong nutrient solutions in the presence of differing amounts of phloroglucinol. Oct. 10, 1914-Jan. 19, 1915.



$$I = \frac{M}{100} \times \frac{1}{5^6}$$

FIG. 17. Average dry weights of ten series of pea plants grown in strong nutrient solutions in the presence of differing amounts of orcinol. Oct. 15, 1914-Jan. 21, 1915.

N.B. Upper curve = mean dry weight of plants which made fair recovery. Lower curve = plants which made poor recovery.

moulds had appeared. Curiously, though, the shoots were not checked at the beginning and for the first week their growth almost equalled that of the controls, but this initial spurt was not maintained, as after a fortnight no further progress was made. Nevertheless the shoots did not wilt or die at all easily, as nearly a month passed before they began to get flabby. Meanwhile the moulds on the roots made rapid progress, and ultimately no signs of life remained in the plants.

With rather lower concentrations, M/100 ×  $\frac{1}{5}$ , the roots were injured from the first, though they were not killed. They were discoloured and made poor laterals, which remained short and stubby for some time. Growth was at a standstill for a period, but after the lapse of two months new laterals appeared, which remained short in some cases and elongated into normal rootlets in others. This continued to the end, and the ultimate effect on growth is shown by the two parts of the curve in Fig. 17, which

shows the mean dry weight of those plants which made a good recovery, and also of those which hung back to the last. Although the roots were injured directly they entered the solutions, the shoots were not affected at first and were as good as those of the control plants. After a time the shoots began to feel the deflection of the roots, and as insufficient nutriment was supplied to them their growth suffered, though they remained green and healthy all along. The improvement in root growth was strongly reflected by that in the shoots, and the plants made rapid headway when once root recovery had set in. Some mould appeared on the roots at an early stage, but did not seem to interfere with recovery.

With the next lower concentration,  $M/100 \times 1/5^2$ , the toxic action was much less marked. For a little time the root laterals were rather short and somewhat discoloured, and before very long some degree of distortion appeared, but later on growth became more normal and the plants approximated in appearance to the controls.

All the lower strengths of orcinol had little or no effect upon growth, as the plants compared very favourably with the controls.

#### Barley.

A comparison of the curves for barley and peas shows that barley is more sensitive to the action of orcinol, as so often happens with these poisons. The highest concentration,  $M/100$ , probably

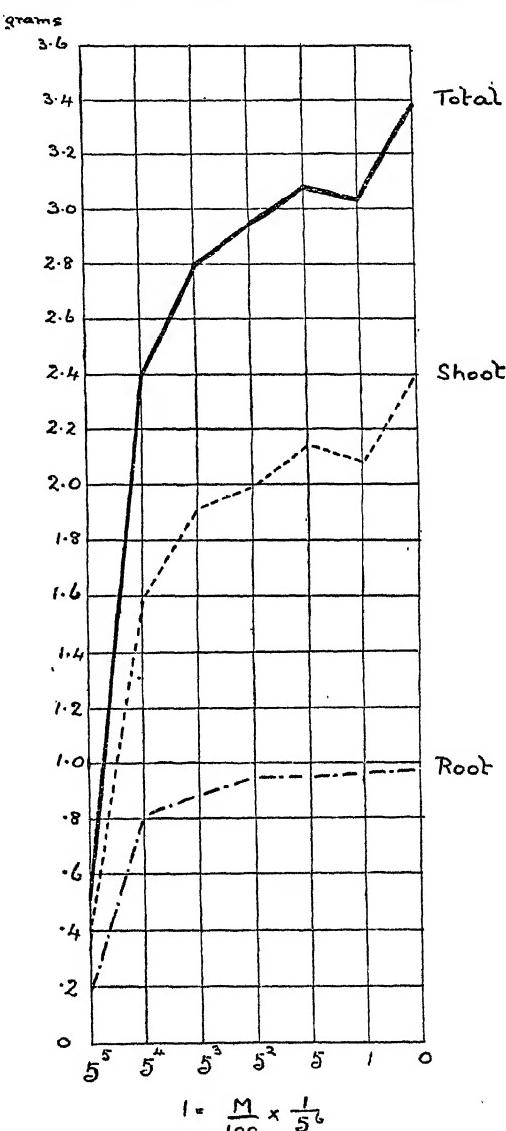


FIG. 18. Average dry weights of ten series of barley plants grown in strong nutrient solutions in the presence of differing amounts of orcinol. Mar. 27-May 17, 1915.

killed the plants immediately, although the shoots grew just a very little during the first three days. The leaves soon began to die down from the tips and the plants quite shrivelled up within three or four weeks. No mould appeared in their case, though with peas such a strong growth was observed.

With  $M/100 \times \frac{1}{5}$  growth was checked at once and for about a fortnight little progress of any kind was made. The leaves kept their healthy green colour, but the stems became purplish. After the plants had marked time for about four weeks the roots began to make slight attempts to put out laterals, but the shoots did not respond at once. Lateral formation proceeded apace and the roots became very full and bunchy, but very few of the laterals ever entered the solutions. Only in three plants was the reluctance overcome, and in these cases the roots were rather long and thick. As a result of this reluctance on the part of the roots the shoots were unable to make as much improvement in growth as might have been expected, and the ultimate result was that the plants were very variable, so that the average dry weight does not in this case convey a true idea of what actually happened.

The toxic effect was still marked with the next strength,  $M/100 \times 1/5^2$ , as for nearly three weeks the root growth was not much better than it was with stronger orcinol. When once lateral formation did make a start, however, progress was rapid. Thick rootlets were pushed out into the solutions, and these were eventually accompanied by more normal thinner roots bearing fine laterals. In spite of all the progress the initial check was never fully overcome and the average dry weight of the plants was considerably behind that of the controls.

Even with a lower strength of orcinol,  $M/100 \times 1/5^3$ , the roots were rather adversely affected at first. For a few days this was not evident, but then the growth of both roots and shoots fell behind that of the controls, although later on most of the leeway was made up.

An interesting feature of the action of orcinol on barley is that at the time of cutting it appeared that there was a decided stimulation with some of the concentrations below those discussed above. This appearance, however, was not borne out by the dry weights, the balance being in favour of the control plants. This apparent stimulation was not observed in the case of peas.

#### SUMMARY OF RESULTS.

1. The general action of the various phenols upon barley and pea plants grown in water cultures is very similar, though the individual substances exercise their specific action at somewhat varying concentrations. In every case a solution containing one per cent. of the molecular weight ( $M/100$ ) of the phenol proves to be fatal, and usually death occurs within

a very short time after the plant comes in contact with the solutions. Occasionally, as with resorcinol and orcinol on peas, the shoots continue to make a certain amount of growth for a few days, even though the roots are killed. Apparently the toxic principle in these cases is not conveyed to the leaves at once, so that they are able to grow for a time at the expense of the food stored up in the seeds. More usually, however, the growth of the shoots is checked simultaneously with that of the roots, though the leaves retain their green colour for quite a long time before they wilt.

The difference in the relative toxicity of the phenols is well shown by the action of solutions one-fifth as strong as the above ( $M/100 \times \frac{1}{5}$ ). Marked toxic action is evident at the first in every case, and the roots are often killed and discoloured. *o*-Cresol, pyrocatechol, and pyrogallol kill peas outright at this strength, but with the other substances the roots make an attempt to right themselves after some time has elapsed. New laterals are pushed out, which frequently refuse to enter the solutions, so that the recovery is only partial. *m*-Cresol admits of only very slight recovery with peas; *p*-cresol, phenol, and phloroglucin permit rather more; while with resorcinol and orcinol quite good root development is able to take place ultimately, with which a corresponding improvement in the shoot growth is associated. Barley is more sensitive than peas, as recovery seldom takes place, and even with resorcinol and orcinol the roots make very little improvement.

The lower strength,  $M/100 \times 1/5^2$ , also shows clearly the difference in the action of the phenols, the range of variation being considerable. In nearly every case an initial check is evident, but the degree of injury varies very much. Resorcinol at this strength has very little effect on peas, as growth is fairly good from the beginning; and with orcinol, too, strong growth is made. Phenol makes the roots bumpy when they put out laterals, but the recovery is so complete that the plants make nearly as much dry weight as the controls. Recovery is variable in amount with most of the other substances, but pyrocatechol is so poisonous that very little growth is made up to the end. Barley behaves in much the same way as peas, though owing to its sensitiveness the recovery is not always so complete as in peas.

Lower concentrations of all the poisons do not seem to exercise injurious action on growth.

2. The root recovery observed in strong solutions suggests that in these cases the poison acts largely by suspending the activities of the plants, paralysing it without killing it outright. Consequently, when the paralysing effect wears off or when the concentration of the solution is somewhat reduced by oxidation (in cases where this occurs), then the plant reasserts its vitality, struggles to put out lateral roots, and frequently succeeds so well that fairly good growth is eventually made.

3. No signs of stimulation have been observed with any of the phenols

tested, except that in the presence of dilute solutions of orcinol barley plants appeared better than the controls before they were cut. This appearance was not corroborated by the dry weights.

4. When the plants were killed by high concentrations of the phenols, moulds usually appeared very rapidly on the dead roots and on the solutions. Phenol itself, however, exerted an antiseptic action for a long time and no mould appeared until towards the end of the experiment, while the three cresols were still more antiseptic and did not allow of any mould formation at any time. This only held good with very strong concentrations, as moulds did appear in rather weaker strengths in which the roots were injured. Where no root injury occurred no moulds grew in any case.

# Studies on the Embryo Sac and Fertilization in *Oenothera*.

BY

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With Plate VII and fourteen Figures in the Text.

THE present paper deals with the behaviour of the gametophytes and fertilization phenomena in *Oenothera nutans* and *Oe. pycnocarpa* as well as their hybrids; these two species were formerly included in *Oenothera biennis* until Atkinson and Bartlett (2) separated them as distinct species. The materials were collected during the summers of 1911 and 1912 in the Botanic Garden of the Cornell University, Ithaca, New York, U.S.A., where this work was commenced, and after the writer's return to Japan the work was carried out in the Botanical Institute of the Tokyo University, and completed in 1915.

The embryo sac and fertilization phenomena of *Oenothera* were already studied by Hofmeister so long ago as 1847 (26), but he could not so fully make out detailed structures of the embryo sac as we do now. He published, however, a figure of tetranucleate embryo sac of *Godetia rubicunda*, a closely related species, and later in 1849 he gave a full description thereof (27). The investigations, to which the modern cytological methods were applied, are of comparatively recent date. In 1908 Geerts (20) published a brief account of the development of the embryo sac and fertilization in *Oenothera Lamarckiana*, which was followed by a detailed paper which appeared in the next year (21). On the other hand, Modilewski (42) independently studied the same subject, and concisely described various cytological features observed not only in *Oe. biennis*, but also in some other plants belonging to the Onagraceae. The results obtained by these two investigators agreed in the essential points that the embryo sac is tetranucleate, and that one pole nucleus and the whole antipodal apparatus are absent. Renner (55) was the third who made a careful study of the embryo sac, fertilization, and subsequent nuclear divisions in *Oenothera biennis*, *Oe. Lamarckiana*, *Oe. muricata*, as well as their hybrids, and made some additional contributions, besides affirming the absence of the melogony

which was reported by Goldschmidt (23) as having occurred in those plants. Lately Werner (73) published a paper on the embryo sacs of *Oe. biennis*, *Oe. Lamarckiana*, and some others, and she discusses in the same paper some ecological relations existing between the gametophyte and ovular tissues. Besides, there are a considerable number of papers dealing with the reduction phenomena in *Oenothera*, which lie beyond the scope of the present paper.

Although the nuclear phenomena of the gametophyte and fertilization of *Oenothera* seem to have been fully described in the papers referred to above, there still remain not a few points which require a careful investigation. The present work was therefore undertaken in order to get an accurate idea about the fertilization, especially with special reference to the behaviour of the synergids at the time of the attack of the pollen-tube. In the course of the present investigation, the materials selected were found very suitable for the purposes.

Several fixing reagents were tried, but Bouin's solution as recommended by Gates (19) was found to give the best result. Some good results were also obtained by using Gilson's fluid, recommended by Renner (55), while Flemming's fluids gave bad results, and Juel's fluid proved still less satisfactory. Sections were cut 8-12  $\mu$ , sometimes 20  $\mu$  in thickness. Flemming's triple stain was applied, but very good preparations were obtained by staining with safranin followed by light green, saturated in clove oil. The latter method, which was recommended by Blackman and Welsford (4), was used to the greatest advantage for any developmental stage of the embryo sac, especially for the structures of the egg apparatus, before and after the fertilization, on account of its transparency and clearness, good differentiation of the cell-wall, and the capacity of showing a fine display of the minute plasmic structure. Heidenhain's iron-alum-haematoxylin, Congo red, and orange G were also used for certain special purposes.

Before commencing the study of the embryo sac, the number of the chromosomes in each species and hybrid were counted in the pollen mother-cells just in the reduction division as well as in the dividing cells in the young ovular tissue; 7 and 14, as expected for  $x$  and  $2x$  number, were respectively found in them. The pollen and ovular structures were found to be also quite identical in one species with any other of these plants under consideration.

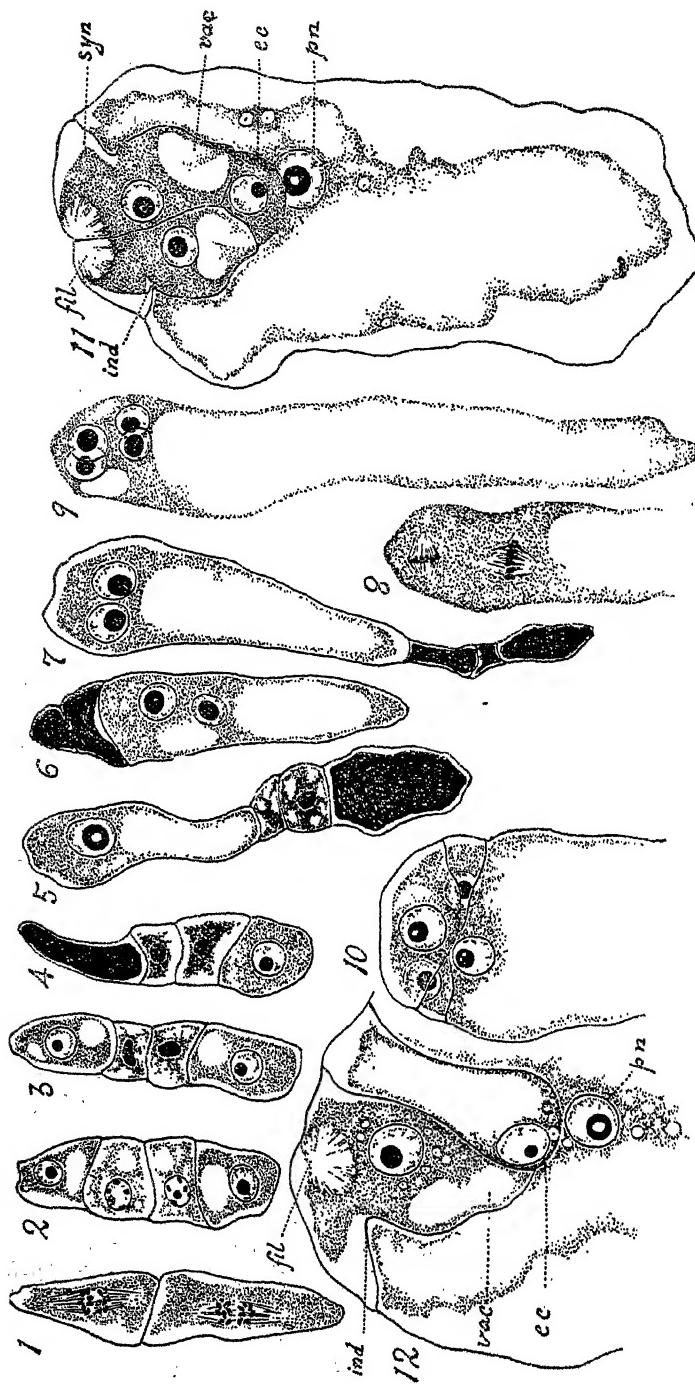
#### EMBRYO SAC AND ITS DEVELOPMENT.

The meiotic phenomena of the embryo sac mother-cell of *Oenothera* have been repeatedly studied by several investigators, as for instance Geerts (21) in *Oe. Lamarckiana* and Davis (15) in *Oe. biennis*. The investigation of those interesting phenomena is, however, excluded in the present paper.

An axial row of four megasporangia is formed by two successive divisions of the embryo sac mother-cell. Text-fig. I, 1, illustrates the early anaphase of the homotypic division, in each nuclear plate of which seven chromosomes are clearly visible. Having finished the tetrad division, the nuclei of the micropylar and chalazal megasporangia soon enter into a resting condition, while those of two intervening ones still remain in telophase surrounded by a thin layer of protoplasm (Text-fig. I, 2). Text-fig. I, 3, shows a little advanced stage, the uppermost and lowermost ones being enlarged by vacuolization, and the two intervening ones at the same time disintegrating. Then one of the survivors, whether micropylar or chalazal, becomes functional (Text-fig. I, 4, 5). According to Geerts (21), in *Oe. Lamarckiana* the uppermost megasporangium is functional; the same was also reported by Modilewski (42) for *Oe. biennis*, while Davis (15) stated that in *Oe. biennis* often the uppermost, but sometimes the lowermost one develops. On the other hand, Werner (73), who studied *Oe. biennis*, *Oe. Lamarckiana*, and several other species of *Oenothera*, obtained the same result as reported by Geerts and Modilewski. In the species used for the present investigation those phenomena just mentioned above equally happen; the fact can quite easily be seen if sufficient number of preparations are examined. Both the uppermost and lowermost ones often simultaneously develop, as will be fully described farther on.

The primary embryo sac cell enlarges and the nucleus passes to the upper pole, the lower part being nearly occupied by an enlarging vacuole (Text-fig. I, 5). The nucleus divides twice (Text-fig. I, 6, 7, 8, 9); the spindles of the last division are, if the available space permits, as observed in many cases, perpendicular to each other (Text-figs. I, 8, and III, 10). The daughter nuclei derived from the upper nucleus are destined to become those of the synergids, while those from the lower nucleus become the egg and pole nuclei. Text-fig. I, 10, represents a section of a young embryo sac, in which the arrangement of two sets of the nuclei are clearly shown. The tetranucleate embryo sac is attained in such a way.

Text-fig. I, 11, shows a mature typical embryo sac of *Oenothera*, in which the egg-cell (*e.c.*) is situated between and just back of the synergids (*syn.*), and only the lower part of the former peeps through the space between the free ends of the synergids. The upper free surface of each of the synergids is horizontally notched by an indentation and presents a visor-like appearance (Text-fig. I, 11, 12, *ind.*). Neither Modilewski, nor Geerts, nor Werner mentions or figures the indentation of the synergids in *Oenothera*, but Renner's illustrations somewhat suggest its presence. The indentation of this kind has been either reported or delineated for several other plants since Strasburger's description for *Santalum album* (66); for instance, in *Helianthus annuus* by Nawaschin (46), in *Parnassia palustris*, *Saxifraga ligulata*, *S. Sponheimica*, *S. cordifolia*, *Heuchera broxioides*, and *Drosera*



TEXT-FIG. I. 1. Embryo sac mother-cell in homotypic division; seven chromosomes are clearly shown in each nuclear plate. 2. Tetrad; both micropylar and chalazal megaspores showing further growth. 3. Two middle megaspores show signs of disintegration. 4. Micropylar megasporite disintegrating. 5. Chalazal megasporite disintegrating, while the micropylar one is developing still further. 6. Chalazal megasporite in binucleate stage, having disorganized sister spores on the upper end. 7. Developing micropylar megasporite with two nuclei and a large vacuole at the upper part. 8. Metaphase of the second division of megasporite nuclei. 9. Tetranucleate stage. 10. Immature embryo sac. 11. Mature embryo sac; synergid (*syn.*) beneath the nucleus. 12. Egg-cell (*cc.*) and large vacuole (*vac.*) beneath the nucleus. *cc.*, egg-cell; *pn*, pole nucleus. 12. Embryo sac cut at right angles to the one above, showing the whole of 3, 5, 6, 10, *Oenothera pycnoarpa*; 1, 7, 8, 9, hybrid '*pumella*'; 2, 4, 11, 12, *Oe. nutans*.

*rotundifolia* by Pace (51), and in *Epilobium angustifolium* by Modilewski (42), whose figures are, however, not very clear. The writer has had an opportunity to observe it in the last-mentioned plant (Text-fig. VIII, 4) and also in *Circaeа quadrifolata* (Text-fig. VIII, 5), as well as in *Godetia sp.* (Text-fig. VIII, 1 a, 1 b), though 4 is not so good as 1 for illustration. The indentation which is associated with the filiform apparatus, though the reverse is not always the case, has been apt to be overlooked by many investigators, probably owing to the fact that its morphological or physiological significance is scarcely understood.

The apical part of the synergids is occupied by the filiform apparatus, which is a solid mass of a conical shape, and is perforated by a number of minute canals which arise on the basal part and converge towards the apex, as fully described by Habermann (25). As its development had not been faithfully followed, it was quite impossible to fully make out the plexus of immense canals. Only in some cross-sections stained with gentian violet, quite a number of minute pores were clearly made out, which must be the cut ends of the canals. 11, 12, *fil.*, Text-fig. I, are intended to show an external appearance of these canals. The following has been compiled to supplement Habermann's list of the plants in which the filiform apparatus is found: *Actaea spicata*, *Podophyllum peltatum* described by Huss (30), *Helianthus annuus* illustrated by Nawaschin (46), *Garcinia Kydia*, *G. Treubii* figured by Treub (71), *Parnassia palustris*, *Saxifraga ligulata*, *S. Sponheimica*, *S. cordifolia*, *Heuchera broxoides*, *Drosera rotundifolia*, *Atmosco texana*, and *Gyrostachys gracilis* described by Pace (51, 51 a, and 52), *Myricaria germanica* by Fristendahl (18), *Butomus umbellatus* by Holmgren (29), *Trifolium pratense* by Martin (40), *Myosurus minimus* by Tchernoyarow (70), *Ottelia lancifolia* by Palm (53 a), and finally *Godetia sp.*, *Gaura Lindheimeri*, *G. parviflora*, *Epilobium angustifolium*, and *Ludwigia prostrata* observed by the present writer, as shown in Text-fig. VIII, 1 a, 1 b, 2, 3, 4, 11. In the mature sac of *Oenothera* and some other plants examined, all belonging to the Onagraceae, a large vacuole usually appears below the nucleus in each of the synergids, which is clearly figured in Text-figs. I, 11, 12, *vac.*, and VIII.

The pole nucleus (*p.n.*), which is almost in contact with the oosphere, increases markedly in size, and is provided with a large nucleolus. As a rule, in the eight-nucleate embryo sac, the secondary embryo sac nucleus is very voluminous, owing, of course, to the fusion of the two polars; but in the case of *Oenothera* the single polar without any additional nucleus acquires a conspicuously large volume. This fact suggests that the polar shares the nutritive function, acting as the antipodal, though the chromatic condition of the nucleus is too normal for the one in such an active condition.

In the nucleolus of the polar, one or several vacuoles are always present, which are often replaced by a crystalline structure, and the same is

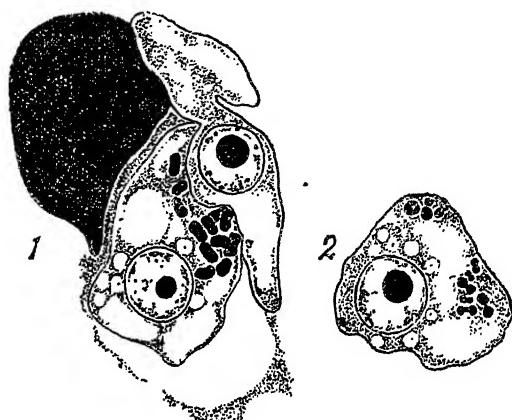
the case with the synergid nucleus. It may perhaps be the proteid crystalloid, though its characteristic reactions could not be brought about by chemical reagents, due to the influence of the fixing fluid. It is, however, interesting to notice the fact that all transitional forms from a spherical vacuole to the crystalloid may be followed; anyhow those structures may need a further investigation. The occurrence of the crystal in the nucleolus has been described by Leitgeb for *Galtonia candicans*, and later by Digby (16) in the same plant, who states that the crystal was probably originated from the nucleolus, and appeared to stain with chromatin dyes. Reed (56) is another investigator who found a similar substance in *Allium Cepa*; according to this author such structures were observed during the development of the spireme, at the end of which they disappeared and seemed to be stained no longer. Dr. Kuwada personally informed the writer that he also found it in the nucleolus of the pollen mother-cell of *Zea Mays*.

Text-fig. I, 12, is another view of a similar embryo sac, cut at right angles to the median line of the embryo sac represented in Text-fig. I, 11. The whole of the oosphere is represented, but one of the synergids is left in another section. Here both the filiform apparatus (*fil.*) and the indentation (*ind.*) of synergids are also clearly shown. A large vacuole occupies nearly the whole space of the egg-cell, in the lower end of which the nucleus lies against the cell-wall. Generally the nucleus of the egg-cell is smaller than that of the synergid, though such a relation is not well shown in this figure. Members of the egg apparatus are clearly separated by a distinct cellulose membrane from one another as well as from the wall of the embryo sac. The lower end of the oosphere is peculiar in this respect, that it possesses neither cellulose nor pectin membrane so far as is indicated by chemical reagents. Plasmolysis does not occur in this portion, while it is clearly discernible in the remaining part; the lower end may probably be bordered by a plasma membrane. As clearly shown in the figure, the apical end of the embryo sac is occupied by the synergids, and the egg-cell hangs down from a position at a distance below the terminal end of the sac. Spherical starch grains were generally found scattered in the plasma sheet of the sac as well as in the lower part of the oosphere, but in a few cases they have been observed as occurring also in the synergids (Text-figs. I, 12, III, 15; Pl. VII, Fig. 9, *b*). Proteid and glucose were detected in the vacuole of the synergid by Habermann (25), who concluded that such proteid and hexose flowed out through the canals of the filiform apparatus, causing a chemotropic reaction of the pollen-tube. The absence of starch grains in the synergid seems to be correlated with the presence of glucose in the vacuole, the glucose being a mere disguise of the starch.

Among over 500 selected preparations which were closely examined, four sections were found in which small rod- or biscuit-formed structures

were observed lying in the egg-cells. The materials for these sections were partly fixed with Bouin's fluid and partly with Benda's fluid.

1, 2, Text-fig. II, which were drawn from the preparations stained with iron-alum-haematoxylin, illustrate two cases in which these structures are found. In the first figure the egg apparatus is shown, in which the synergid darkly shaded is plumped by the attack of the pollen-tube, while the other is shrinking. Between those is situated the fertilized egg-cell, two synergids containing a small number of the structures in question, which are scattered between the cell-wall and the right-hand side of the nucleus. The second figure represents a section of the oosphere showing two groups of those structures, each small body of which makes a pair and looks as if the couple has just been derived from a common source by division. Unfortunately, the specimens are not sufficient for a more detailed examination. But judging from all the appearances these small bodies seem to be something like chondriosomes.



TEXT-FIG. II. 1. Hybrid 'pycnelia'. Egg apparatus showing chondriosome-like structures in a fertilized egg-cell. 2. *Oenothera nutans*. Oosphere containing two lumps of chondriosome-like structures besides some starch grains. Both figures  $\times 1,300$ .

#### DOUBLING OF EMBRYO SAC.

Among the Angiosperms the occurrence of the excess embryo sac mother-cell is commonly known. *Oenothera* is not exceptional in this respect. Geerts (21) reported such a case in *Oe. Lamarckiana*; and the same was also described by Täckholm (68) for *Oe. biennis*, in which two rows of the tetrads occurred lying side by side. Besides, he met with four or five definitive archesporial cells in the closely related genus *Lopezia*, and always several mother-cells in *Godetia* and in *Clarkia* (69). Text-fig. III, 1, shows two embryo sac mother-cells in synapsis lying one upon the other. Text-fig. III, 2, represents two rows of the tetrads, one set normally developing and the other showing a sign of disintegration. Several other cases have been studied, with a result that only one of the mother-cells or its derivatives is functional.

Many cases have been reported where several primary embryo sac cells, derived from the different mother-cells, start forming the embryo

sacs ; but, as to the twin sacs, only few examples have been given. Coulter and Chamberlain (13) summarized the cases known at that time : two ripe sacs in *Lilium candidum*, *Salix*, *Juglans cordiformis*, *Delphinium*, and *Senecio*, and two or more sacs in *Fagus*, *Corylus*, and *Carpinus*. Since their publication several other examples have been reported : mature twin sacs in *Ulmus americana* by Shattuck (61), in *Nelumbo lutea* by Cook (11), in *Lychnis alba* × *L. Flos-cuculi* by Compton (9), in *Smilacina racemosa* by McAllister (41), in *Peperomia hispidula* by Johnson (32), in *Gastrodia elata* by Kusano (36), and in *Godetia Whitneyi*, *Fuchsia 'Marinka'*, and *F. 'Émile de Wildeman'* by Täckholm (69). In addition, the following cases have also been reported : three sacs in *Cheiranthus Cheiri* by Schacht (57), two in *Taraxacum officinale* by Schwere (59), the same in *Balanophora globosa* by Lotsy (37). The writer has also found two sacs in *Ludwigia prostrata* and *Jussiaea repens*, the latter case being illustrated in Text-fig. VIII, 9 a, 9 b, 9 c. The above-mentioned mature double embryo sacs have been explained by the previous investigators as having derived from two independent mother-cells, except in *Nelumbo lutea*, in which Cook (11) stated that 'two sacs were found one below the other in the main axis of the ovule. The extra sac was evidently derived from one of the sister megasporangia.' This is the only example of mature twin sacs originated from two sister megasporangia, though we know many examples in which all or some of the sister megasporangia develop as far as either a bi- or quadrinucleate condition is reached, but only one of the original megasporangia becomes a mature embryo sac.

In *Oenothera* studied by the writer the twin sacs commonly occur, sometimes lying side by side (Text-figs. III, 14, 16, and IV), sometimes one upon the other (Text-fig. III, 13, 15). It seems to be the general rule in these plants that of four megasporangia the two interjacent ones first disintegrate, then one of the survivors follows the example. But, if both survivors simultaneously begin to develop, the twin sacs may ultimately be attained. This is just the case with *Oenothera*. No case has been observed, however, in which more than two megasporangia had developed. 3 and 5, Text-fig. III, show two successive stages, the former of which represents two growing megasporangia, and the latter similar ones in a binucleate stage, the micropylar one of which is ready for a tetranucleate state. Text-fig. III, 6, illustrates two developing megasporangia in a binucleate condition, though one of the nuclei in the lower megasporangium is left in the next section. These structures generally give rise to twins, as shown in Text-fig. III, 13, 15. Geerts (21) states that he once observed an embryo sac which contained eight nuclei, and that in the middle portion of the sac a partition line was clearly visible. Though he did not give any figure, the sac observed by him must have been in the same condition as those described above.

In Text-fig. III, 13, the upper sac, which is already attacked by the pollen-tube, is pressing the lower one, while the filiform apparatus is developed

in each of them, though the whole aspect cannot be included in this section. In Text-fig. III, 15, the lower one, whose polar is in the adjoining section, is gaining, and the filiform apparatus are developing in both of them. The dislocation of the megasporangia often takes place in the course of their development (Text-fig. III, 4, 7), resulting in the formation of the condition delineated in Text-fig. III, 17, in which the smaller one appears as if partly embedded in the larger sac. If the above-stated dislocation proceeds farther and the inferior one remains, two embryo sacs attain a parallel position (Text-figs. III, 14, 16, and IV). Often the lower megasporangium remains in uni- or binucleate state, as shown in Text-fig. III, 11, 12. A similar condition has been reported as occurring in a related species, *Lopezia coronata*, and in some others by Täckholm (68, 69). The writer has found the same phenomenon in *Ludwigia prostrata* (Text-fig. VIII, 10); such a case is, however, known to occur in a great many plants belonging to other families. 8, 9, 10, Text-fig. III, show several younger stages which correspond to the cases as shown in Text-fig. III, 11, 12. Text-fig. III, 9, shows one of the intervening megasporangia still surviving, and in Text-fig. III, 10, the upper one is in a tetranucleate stage, while the lower is binucleate, though the nuclei are not visible in this section.

As to the fate of the excess embryo sac, nothing conclusive can be stated, owing to the lack of the material, except that it may disintegrate sooner or later when the ovule is entered by a single pollen-tube, or it may be visited by a pollen-tube (Text-fig. IV), when more than one pollen-tube penetrates the ovule.

In Text-fig. IV, which has been reconstructed from three succeeding sections, ideally cut, the sac on the right-hand side is just before the fertilization, while the other is in contact with the pollen-tube. This figure suggests possibility of fertilization in both of the embryo sacs. Schwere (59) working on *Taraxacum officinale* found a case of twin embryo sacs, each giving rise to a normal embryo; such was also the case with *Gastrodia elata* studied by Kusano (36), in *Lychnis alba* × *L. Flos-cuculi* by Compton (9), and in *Ulmus americana* by Shattuck (61), though in the latter two cases it was not retraced farther up to the embryo sac formation.

The persistency of more than one megasporangium in *Oenothera*, which is situated in the higher rank among the Polypetalae, shows the atavistic inclination of the young gametophyte caused by an evolutional tendency.

#### MALE GAMETOPHYTE.

A full description concerning the development of the pollen mother-cell of *Oenothera* has been given by Geerts, Gates, and Davis; the further growth of the pollen, especially the development of the pollen-wall, has been faithfully followed by Beer (3). The ripe pollen grain, just before shedding, contains a vegetative nucleus (Pl. VII, Fig. 1, v) in the centre

and a generative one (Pl. VII, Fig. 1, g) in the peripheral portion. The former is presented as a spherical chromatin mass showing a sign of disintegration, and embedded in a plasma mass, while the latter is smaller and ellipsoidal, and is surrounded by a thin plasma sheet. The rest of the contents is impregnated with immense numbers of hyaline, fusiform corpuscles (Pl. VII, Fig. 1), which scarcely stain with the usual chromatin dyes, so that they are apt to be overlooked, and the whole contents of the pollen grain are taken for an alveolar plasma mass. These corpuscles are typical fusiform or oblong ellipsoidal in shape, with acute or often obtuse ends, and show the reaction characteristic to starch by treating with iodine solution. Starch grains were often found in pollen grains of many plants; they were spherical, ellipsoidal, or rod-like, but not fusiform as in the case of *Oenothera*, although Guignard (24) reported that there were fusiform starch grains in the pollen and pollen-tube of *Najas major*. Thus, it was found necessary to test whether they were really starch grains or not. But, having no fresh material at hand, *Oenothera odorata* was used for this purpose, whose pollen grains are quite identical with those of *Oe. pycnocarpa* and of *Oe. nutans* in every minute respect. The fresh pollen grains were crushed under the cover-glass, and several reagents were applied upon them. The result was as follows: By adding iodine solution the corpuscles in question turned blue, while by heating they became decolorized and swollen; they swelled also when treated with chloral hydrate; they disappeared after standing 6–20 hours in 1–2 per cent. solution of 'Takadiastase', keeping them in 60° C. These reactions leave no room for doubt that they are no other than starch grains.

The pollen-tube reaches the embryo sac at least in 48 hours after pollination. In an experiment, some pollen-tubes have arrived in the ovules in 53 hours and 30 minutes; in this case some eggs were already fertilized or just being done, but some embryo sacs were laid free from invasion of tubes. The plasm of the pollen-tube contains a large number of starch grains which are fusiform, ellipsoidal, or spherical, but not so uniform as observed in the pollen grain; this fact suggests that starch grains undergo some chemical changes, owing probably to the use as nutrition of the tube. Pl. VII, Fig. 2, illustrates the lower portion of the tube travelling through the stylar tissues, and carrying a disintegrating tube nucleus (*v*) and two male nuclei (*m.n.*) which are in the last telophase. In the upper portion of the tube, though not shown in the illustration, a series of stoppages or cross septa are laid down, which showed the reaction characteristic to callose, being stained red with corallin-soda. Nodal swellings and branching of the tube were often observed; the latter case was always found in the micropylar portion, and made it difficult to distinguish whether two different tubes or a pair of branches were present. The wall of the tube is never found of a uniform thickness, particularly in the apical portion.

Two sperm nuclei were often found in the lower end of the tube, which lay just upon the embryo sac, but the vegetative nucleus was not associated with them, though Modilewski (42) observed and delineated both. Much attention was focused upon this question, but no vegetative nucleus happened to be found there at all. It may have been suspended at one of the nodal swellings of the tube, because it is often the case that the tube nucleus is found staying in such a place. Or it may have become absorbed in the tube plasm before it reached the embryo sac. The male nucleus is ellipsoid or ovoid, containing few chromatic masses besides one to several small nucleoli, and is surrounded by a distinct plasma sheath which presents itself as elliptical or lenticular in form (Pl. VII, Figs. 4, 5, 7). In some cases both of the nuclei are found embedded in a common plasma sheath (Pl. VII, Fig. 6). It is interesting to notice that a greater quantity of plasm is found in front of than behind the male nucleus. This fact suggests that the nucleus is dragged by the plasm, causing the movement of the male cell towards the embryo sac. Blackman and Welsford (4) noticed, in *Lilium*, that one of the male nuclei which was destined to fuse with the egg nucleus was smaller than the other. This was not the case in *Oenothera*, and both the nuclei are nearly equal in size. Neither Modilewski (42) nor Geerts (21) has described or figured the plasma sheath in *Oe. Lamarckiana* and in *Oe. biennis*. In a paper on *Myosurus minimus*, recently published by Tchernoyarow (70), there are many good illustrations of male nuclei, embedded in a distinct plasma sheath in the pollen-tube as well as in the plasma mass, which flowed out of the synergids, and spread upon the oosphere. According to this observer, the male nuclei shed the plasma sheath before the fusion, as in the case of *Oenothera*. The cases illustrated in his figures agree very well with those observed by the writer in *Oenothera*, except that two male nuclei always lie in a common plasma sheath.

#### FERTILIZATION.

On reaching the apical end of the sac, the pollen-tube proceeds to the upper part of the synergid, but never directly passes to the egg-cell, though the latter is situated so close to the tip of the former. It has been observed only once that the tube happened to come to the upper end of the egg-cell, but, without visiting the latter, turned the way to the apex of the filiform apparatus. On arriving at the synergid the pollen-tube pierces the filiform apparatus first of all, and generally makes its way through the border line of two adjoining apparatus (Pl. VII, Figs. 8, 10). As stated above, Habermann's conclusion is that the chemotropic reaction of the pollen-tube is induced by glucose and proteid, which are poured out through the canals of the filiform apparatus from the store of the vacuole in the synergid. It just explains the behaviour of the pollen-tube of *Oenothera*. In some cases the end of the tube expands just upon the filiform apparatus, and the

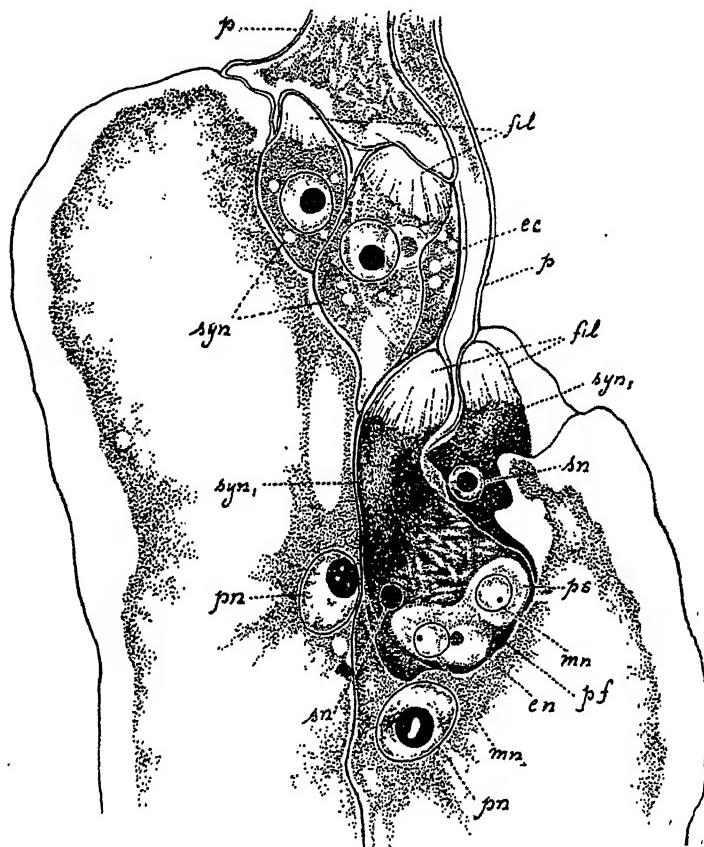
narrowed tip penetrates the apparatus (Pl. VII, Figs. 12, 17); in other cases the tube ramifies at the contact surface, reminding one of the pseudopodia of Amoebae or clasping fingers, and some of the branchlets proceed farther (Pl. VII, Fig. 18). In such a case it often causes dislocation of the filiform apparatus (*fil.*), which subsequently becomes smothered by the contents of the attacked synergid (*syn.*) flowing out. The tip of the pollen-tube has no special apparatus for pouring out its contents. A small pore seems to be in preparation for a time, probably by the action of certain enzymes, such as cytase and pectinase, secreted by the male cell, and acting upon cellulose and pectic substance, of which the wall of the tube and filiform apparatus are composed. Though it is a mere supposition, the same enzymes may attack next the filiform-apparatus for the passage of the tube, and then act upon the wall of the invaded synergid, which thus becomes actually perforated, as will be described later on. The contents of the tube are poured into one of the synergids (Pl. VII, Figs. 10, 12), or often into both of them (Pl. VII, Figs. 8, 11, 14, 17; Text-figs. IV, VII, 3), probably owing to the accidental position of the tube end, so that sometimes it causes the enzymes to act upon the walls of both synergids.

The plasm containing starch grains in the pollen-tube is poured into the attacked synergid (*syn.*) as shown in Text-fig. VII, 1-3; Pl. VII, Figs. 8-12, 17, 18; especially Fig. 17 shows the critical moment of the intrusion of those expelled grains. For this reason the grains serve as a clue to trace the contents of the tube. Iodine solution is the best reagent for this purpose. The intrusion of starch grains from the pollen-tube has already been mentioned and illustrated as occurring in *Najas major* by Hofmeister (28) in 1858. He also observed so early as 1847 the migration of starch corpuscles from pollen grains to pollen-tubes in several other plants, especially in *Oenothera*, *Godetia*, and *Boisduvalia* (26).

The invaded synergid becomes much swollen with the additional protoplasm, and the contents suddenly get chromatic, but starch grains inside are still perceptible. The wall of the synergid bursts, with the result that the mixed contents flow out upon the lower part of the oosphere, which is thus covered by them for a while. Pl. VII, Fig. 9 *a*, represents a side view of an attacked synergid (*syn.*); the contents (*p.f.*) are just going to spread over the oosphere (*e.c.*) through the several rents (*r.*) on the lower side. Fig. 9 *b* is another section obtained from the same sac, showing the other sound synergid (*syn.*) and the egg-cell (*e.c.*), the latter of which is just ready for fertilization. Text-fig. IV clearly illustrates an advanced stage: in the right-hand one of the twin sacs, the oosphere is totally covered with the contents (*p.f.*) of the invaded synergid (*syn.*), while the disorganized synergid nucleus is seen as a spherical chromatic mass (*s.n.*), and two male nuclei (*m.n.*) embedded in a hyaline plasma sheath (*p.s.*) are clearly visible, through which the egg nucleus (*e.n.*) is discernible. Figs. 14 and 16, Pl. VII, represent

some more examples of the same stage as the latter. Fig. 13 illustrates a similar case, showing two male cells (*m.n.*) and egg nucleus (*e.n.*) in the oosphere, the lower end of which has been cut off obliquely.

It appears that two sperm cells always pass through the synergid before they reach either the egg-cell or the pole nucleus, although no direct proof

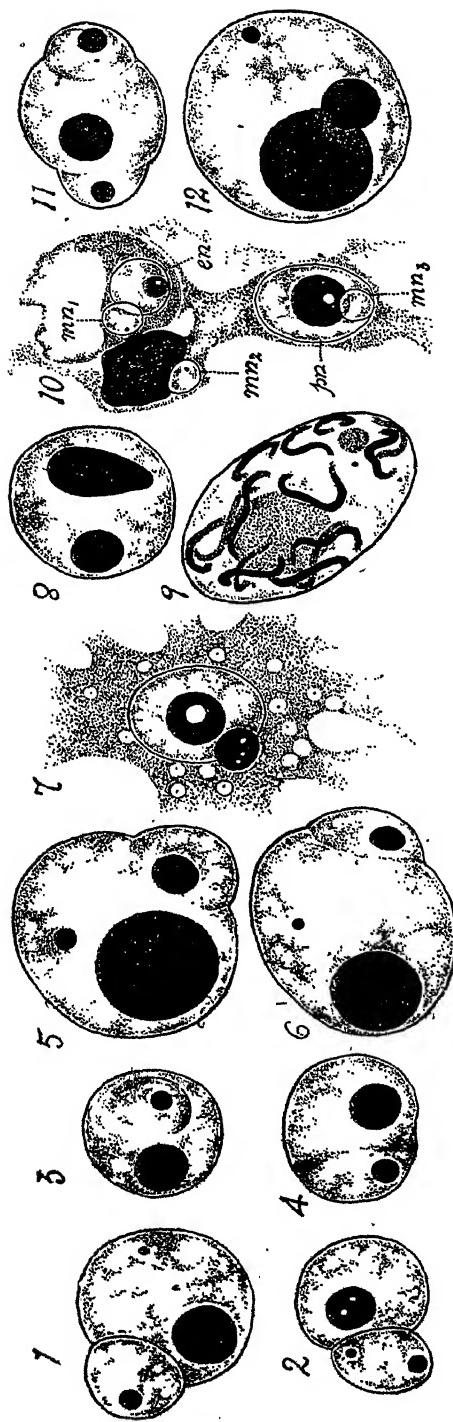


TEXT-FIG. IV. Twin embryo sacs of *Oenothera nutans* fertilized by *Oe. pycnocarpa*, both of which are attacked by pollen-tubes (*p.*); the right-hand one has already been penetrated by the tube and plasma flow (*pf.*) from the synergid (*syn.*) is spreading over the oosphere; on the lower end of the latter two sperm nuclei (*mn.*) lie surrounded by a distinct plasma sheaf (*ps.*), through which egg nucleus (*en.*) is visible. *syn.*, sound synergid; *fil.*, filiform apparatus; *ec.*, egg-cell; *sn.*, synergid nucleus; *pn.*, pole nucleus.  $\times 1,300$ .

has been obtained. When perched upon the egg-cell, two sperm nuclei are clearly seen, for each of them is covered with a hyaline plasma sheath, which is stained well with light green, while the poured plasma mass is very chromatic and is stained dark red with safranin. Pl. VII, Figs. 13 and 14, and Text-fig. IV were drawn from the preparations stained with a combina-

tion of safranin and light green, on which every detailed structure is shown up most brilliantly. Fig. 16 is delineated from a section stained with a combination of light green and iron-alum-haematoxylin; the plasma sheath (*p.s.*) stained with the former is beautifully differentiated from the surrounding plasma mass (*p.f.*), which is stained dark with the latter. In Fig. 13 and Text-fig. IV two male cells are lying side by side, while in Fig. 14 one of them is situated a little way down towards the pole nucleus, and through the other male cell the egg nucleus (*e.n.*) is visible. In Fig. 16 only one of them is illustrated. At the moment of the fusion, the sperm nucleus (*m.n.*) bears no plasma sheath (Fig. 15), the latter being probably cast off beforehand. At any rate in *Oenothera* the male products arrive at the female organ as sperm-cells, but not as sperm nuclei. The question whether the sperm nuclei are naked or not has been fully discussed by Strasburger (67), Körnicke (35), Nawaschin (47), and Welsford (75), who agreed as to the absence of the plasma sheath in *Lilium*. On the other hand, Juel (84) has reported that he observed in *Saxifraga granulata* a thin plasma mass, attached on each of the sperm nuclei, which just slipped out of the synergids. Němec (49) has described in *Gagea lutea* that the sperm nucleus is covered with a thin plasma mass lying in the synergid. A similar case has been also reported by Shattuck (61) for *Ulmus americana*, who states that 'the male cells lose their cytoplasm on entering the pollen-tube, and during their journey to the embryo sac are simply elongated nuclei. After entering the sac the nuclei become spherical and begin to gather a small amount of cytoplasm around them.' Nawaschin in 1909 (46), and later in 1915, working with Finn (48), observed in *Juglans nigra* and *J. regia* that two sperm nuclei either in the pollen-tube or embryo sac are always found embedded within a common vesicular sheath, which they state to be the residue of the cytoplasm of the generative cell, though it is cast off before fertilization. Pace (51) is another investigator who gave a description of the occurrence of the granular plasma sheath around the sperm nucleus, when the latter is lying in the synergid, in *Parnassia palustris*. Recently Tchernoyarow (70) stated that in *Myosurus minimus* two male nuclei are enclosed in a common plasma sheath up to the moment of fertilization, when they cast off the cover in the embryo sac. These results indicate that possible existence of such a sperm-cell may be detected, if closely examined, in not a few families of the Angiosperms.

Modilewski (42) has reported entry of the vegetative nucleus into the embryo sac, though no trace of such has been found by the writer in the sac, nor even in the end of the tube just before shedding, as already stated above. Only the degenerating synergid nucleus has often been observed as a chromatic globule in the attacked synergid, as shown in Pl. VII, Figs. 13, 15, *s.n.*, and in Text-fig. IV, *s.n.*. Although it is in a degenerating condition, the vegetative nucleus is quite different from the chromatic nucleus of the



TEXT-FIG. V. 1-4. Various ages of fusion of male and egg nuclei. 5-6. Fusing pole and male nuclei. 7. Disintegrating male nucleus perching upon polar nuclei. 8. Endosperm nucleus with two nucleoli of different size, that suggesting origin of each of them. 9. Prophase of division of endosperm nucleus; fourteen chromatid filaments are clearly differentiated. 10. Three male nuclei; the upper ( $m.n_1$ ) is fusing with the egg nucleus ( $e.n.$ ); the second ( $m.n_2$ ) is in contact with the polar ( $p.n.$ ), while the third ( $m.n_3$ ) lies beneath the chromatic mass. 11-12. Both figures were obtained from the same embryo sac. Fig. 11 shows the egg nucleus uniting with two male nuclei, while in 12 the polar received another male element.

1, 2, 3-6, 10-12, *Oenothera nutans* fertilized by *Oe. pinnatifida*. 7-9, *Oe. lamarckii*. 1-6, 8, 9, 11, 12,  $\times 220$ . 7, 10,  $\times 1040$ .

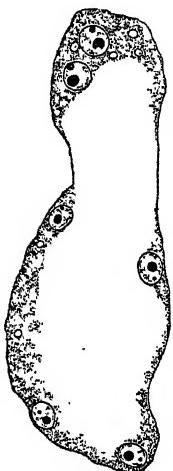
synergid in its general appearance. This relation is clearly perceptible if Figs. 2, 3, v., Pl. VII, are compared with those above mentioned.

The fusing sex nuclei have been observed many times. They come in contact in the resting condition, simply fuse, and give rise to a larger nucleus containing two main nucleoli of different sizes, that derived from the male nucleus being smaller than that from the egg nucleus. 1, 2, 3, 4, Text-fig. V, illustrate their behaviour at the critical moment of the fusion of both elements, and 5 and 6 show the same cases in the polar and sperm nucleus. Attention has been paid to the question of the plasma inclusion between two fusing nuclei, as stated by Brown (5) in *Peperomia Sintensis* or in *Gagea lutea* by Němec (49), but in *Oenothera* no trace of such bearings was found. Sometimes the pole nucleus was found just receiving a disorganizing male nucleus as shown in Text-fig. V, 7.

This fact shows occasional disintegration of the male nucleus, and perhaps subsequent arrest of further growth of the polar or egg nucleus, if it happens to visit the latter.

After the fertilization has taken place, the chromatic plasma coat which covers the egg-cell soon disappears, probably having been absorbed, and the lower end of the egg-cell also acquires a distinct cellulose membrane, which is clearly visible when the plasmolysis of the contents has occurred. Some starch grains are carried out from the synergid by the plasma flow, in which they are found spherical or ellipsoidal but not spindle-shaped. This fact shows that the grains are undergoing some chemical change. The fate of these starch grains has not been followed, but it may be presumed that they become finally consumed.

TEXT-FIG. VI. Section through plasma sheet with sixteen endosperm nuclei, showing two nucleoli in each of them.  $\times 450$ .



The remaining corpuscles in the synergid generally retain their original form and seem to remain unchanged, until they become absorbed together with the shrunk synergid when the fertilized egg begins to develop. The division of the primary endosperm nucleus quickly follows the fertilization, resulting in the migration of two daughter nuclei to both sides of the egg apparatus (Pl. VII, Fig. 10, *end n.*) when the division has succeeded twice. This nuclear position served as a clue to determine whether fertilization has taken place or not. As shown in Fig. 10 and Text-fig. V, 8, generally the divided nucleus still possesses two nucleoli; such nuclear condition is retained through many nuclear generations (Text-fig. VI). The mitotic figures of the endosperm nuclei were often found in the preparations, but no favourable

case to count the number of chromosomes has been observed. Once a nucleus in prophase of the second division was observed, in which fourteen chromatic filaments associated with two nucleoli were found (Text-fig. V, 9). This indicates the diploid nature of the endosperm nucleus. Renner (55) gave a good illustration of the nuclear plate found in the plasma sheet of the fertilized embryo sac in *Oenothera biennis*  $\times$  *Oe. Lamarckiana*, in which fourteen chromosomes are clearly delineated. This is the positive proof of a diploid nature of the endosperm nucleus. The first division of the fertilized egg nucleus occurs after several succeeding divisions of the primary endosperm nucleus; for instance, the egg nucleus remains unchanged, while sixteen endosperm nuclei are found in the plasma sheet.

An unusual case is shown in Text-fig. V, 10; in the upper right-hand side of the figure, a male nucleus (*m.n.<sub>1</sub>*) is shown just fusing with the egg nucleus (*e.n.*), and on the left-hand side the second nucleus (*m.n.<sub>2</sub>*) is shown lying beneath the chromatic plasma mass, while the third (*m.n.*) is just coming into contact with the large pole nucleus (*p.n.*). No shadow of the vegetative nucleus could be found in the sac, of course, so that those just mentioned above must be three sperm nuclei. For explanation of such an aberrant case two alternatives are proposed. The first is that the presence of excess nuclei is brought about by intrusion of two sets of sperm nuclei due to the attack of two pollen-tubes on a single embryo sac, though the fourth nucleus does not happen to be found in the preparation. The second is that it is due to the production of some excess generative nuclei in the male gametophyte. Among the Angiosperms visit of two or more pollen-tubes on a single embryo sac sometimes happens, as stated by Shattuck (61) in *Ulmus americana*, by Juel (34) in *Saxifraga granulata*, by Němec (49) in *Gagea lutea*, by Compton (9) in *Lychnis Flos-cuculi*, by Weinzieher (74) in *Xyris indica*, by Nawaschin and Finn (48) in *Juglans nigra* and *J. regia*, by Tchernoyarow (70) in *Myosurus minimus*, and as described above in *Oenothera* (Pl. VII, Fig. 8), although in the latter case it was often quite difficult to determine whether they represent both ends of a ramified tube or belong to different stocks. Unfortunately in the preparation containing the aberrant figure in question the pollen-tube is lacking, in consequence of an accident while preparing, so that the specimen was not complete enough to afford a decisive conclusion. According to Weinzieher, two pollen-tubes pour out the contents into an embryo sac, but he does not mention anything about the further destiny of the poured contents. Nawaschin and Finn (48) report that two or three sets of male nuclei were often found ejected in a single embryo sac, but not two sperms fusing with an egg. The same was also observed by Tchernoyarow (70). The latter three authors' statements are illustrated by figures clearly delineated. Judging from the above-mentioned facts, especially the clear descriptions given by those three workers, it is reasonable to explain

the fate of the extra male nucleus in the embryo sac of *Oenothera* by the first alternative proposed above, which was also maintained by Němec in the case of polyspermy in *Gagea lutea*.

The second alternative, that is, the production of an extra nucleus in the male gametophyte, may be no less applicable than the first one for the explanation of the case. Several examples referred to the latter case have been reported, one of which is that of *Lilium aurantiacum* examined by Chamberlain (7), who found three male nuclei in the pollen grain. Another is the production of four male nuclei in the pollen-tube of *Ornithogalum* and *Scilla nutans*, studied by Strasburger (65). No such cases have been found in *Oenothera*, but another aberrant case, which will be given below, suggests its probable occurrence. Therefore, though not conclusive, the writer is inclined to explain the case in question by the first as well as the second alternative, until some more conclusive facts are obtained.

Another aberrant case just mentioned is the fusion of an egg nucleus with two male nuclei, while the pole nucleus of the same embryo sac receives another male nucleus, as shown in Text-fig. V, 11, 12. 11 shows that the egg nucleus is fusing with two male nuclei on both sides, and in 12 two nucleoli of different size are found in a large pole nucleus, which is therefore a perfect primary endosperm nucleus. The embryo sac from which these figures were drawn was carefully studied, and it was found that it had received only one pollen-tube. Thus it cannot be denied that an extra male nucleus was produced in the male gametophyte. Němec (49) often observed triple fusion in the egg nucleus in *Gagea lutea*, and proposed several hypotheses for this question, suggesting the origin of a plant possessing triploid nuclei as having been derived from an egg nucleus fertilized by two male nuclei. Though there is some difficulty in arriving at a satisfactory conclusion as to the distribution of the chromosomes at the time of the reduction division in the next generation, the origin of the triploid mutant must be ascribed to a fusion of diploid and haploid germ nuclei, in order to be more rational within the scope of the present knowledge. In this respect, Němec's view seems to be very ingenious, who substituted two male nuclei for the diploid germ nucleus. Some would be opposed to his view, advocating that the dispermic egg cannot give rise to a new plant owing to the early disintegration. But our present knowledge does not permit us to state whether the dispermic egg can develop further or not, and consequently we can neither deny nor affirm it. The opponent must be influenced by some zoological evidence, for an actual development of the dispermic egg-cell has been recorded by several investigators, though some irregularities in the distribution of the chromosomes in further cell division may occur, being often induced by interference of the extra centrosomes. But in the higher plants no such interference does occur even if polyspermy may happen. At any rate, Němec's view seems to be the most natural one among several

hypotheses as yet proposed. Really, triploid mutants in *Oenothera* were reported by Lutz (38), Stomps (63, 64), and Gates (19 a). The first-named observer, having found them in the offspring of *Oenothera lata*  $\times$  *Oe. Lamarckiana* and self-pollinated *Oe. lata*, attributed the origin of such mutants to the union of the reduced male nucleus and unreduced egg nucleus. Stomps independently published the occurrence of a similar phenomenon in *Oe. Lamarckiana*, which he named *semigigas*, and he got also a triploid offspring from *Oenothera biennis* and named it *Oenothera biennis semigigas*. Though these first two investigators did not touch the dispermic problem, the case would be easily and smoothly explained by Némec's hypothesis, which has, however, been suggested by Gates as being less probable than the union of diploid and haploid sex nuclei.<sup>1</sup>

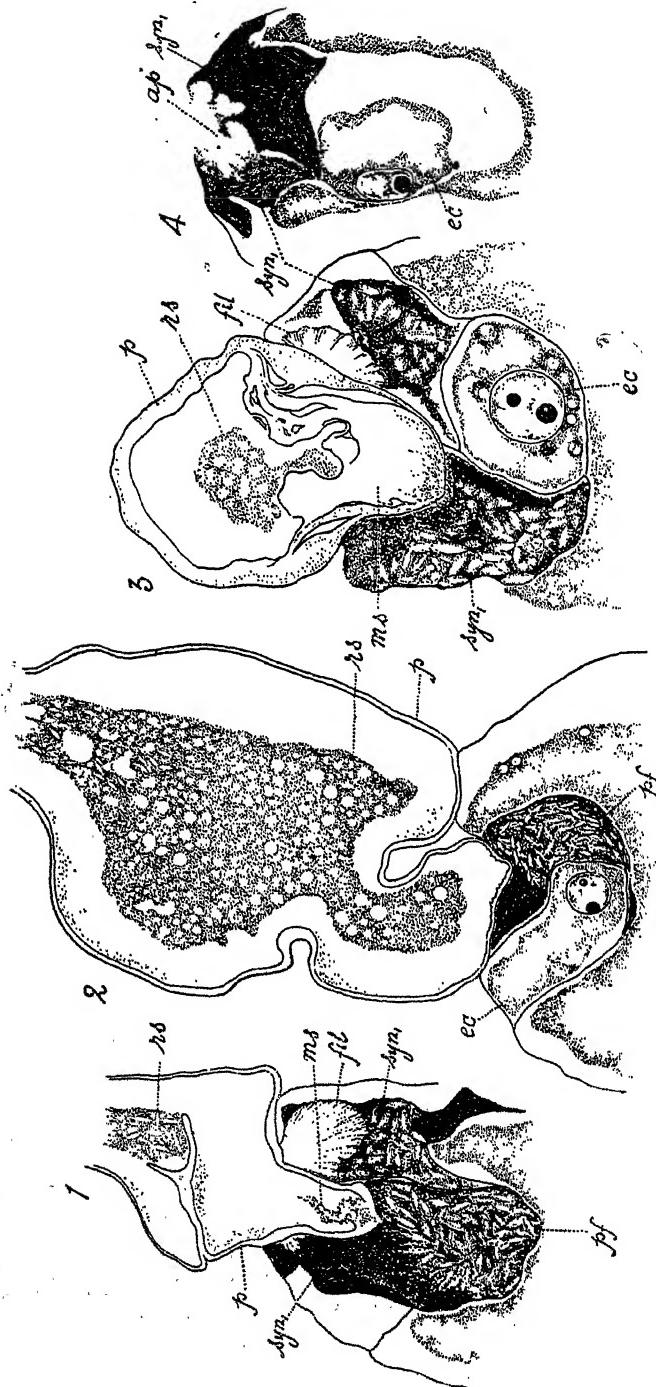
After the ejection of the contents, the opening at the tip of the pollen-tube is closed by some mucilaginous substance (Pl. VII, Fig. 8; Text-fig. VII, 1, 3, *m.s.*) forming the residue (*r.s.*) of the contents of the tube. If there remain any starch grains they gradually disappear, and the contents finally become filled with many globular liquid drops as shown in Text-fig. VII, 2, *r.s.* The filiform apparatus persists for a long time, even when the rest of the synergids is entirely absorbed at the time of embryo formation.

#### CELL WALLS OF EGG APPARATUS.

For the determination of the chemical character of the cell walls of the egg apparatus, a great many microtome sections have been treated with different stains and reagents, and the results obtained are as follows :

*Filiform apparatus.* The filiform apparatus (Text-fig. VII, 1 and 3, *fil.*) was stained red with aqueous solution of Congo red; also it was coloured purplish blue with chlorzinc-iodine. A treatment with freshly made cuprammonia gave the following result. Sections were allowed to stand in this reagent for half a day to 30 hours; the apparatus was then found entirely dissolved, as shown in Text-fig. VII, 4, *ap.*, often leaving some cloudy residue.

<sup>1</sup> After the manuscript was finished, Dr. I. Ōsawa published an interesting account of triploid mutants in the garden races of *Morus* in the Bulletin of the Imperial Sericultural Experiment Station, Japan, vol. i, No. 4, 1916 (Japanese). According to this investigator, in forty-two garden races of *Morus* examined by him, supposed to have been derived from *Morus bombycis*, *M. alba*, *M. indica*, and *M. multicaulis* (?), each of thirty-two races possesses fourteen haploid and twenty-eight diploid chromosomes. And a certain one of the fourteen haploid chromosomes is very conspicuous for its remarkable size. On the other hand, in the remaining ten races he studied, forty-two chromosomes are equally found in the vegetative cell, three of which are always markedly larger than the others. As he did not find such a race that has twenty-eight haploid chromosomes, he is inclined to believe that those ten races just mentioned have not been derived from a hybrid between two kinds of races which possess fourteen and twenty-eight chromosomes respectively, but really descended from a triploid mutant. Although the investigator adopts the prevailing hypothesis, i. e. the union of the haploid and diploid generative cells for the explanation of the origin of those triploid mutants, the case will be smoothly explained by Némec's hypothesis. The triploid mutants in question are all sterile, as Dr. Ōsawa stated, but having been propagated vegetatively, for instance by grafting, so many triploid mutants seem to have been preserved and protected from extinction.



TEXT-FIG. VII. 1. Open end of pollen-tube ('*p.*') in synergid ('*syn.*'), lined with mucilaginous substance (*m.s.*). *fil.* = filiform apparatus. 2. Remainder ('*r.s.*') of tube contents, with some liquid drops. 3. Tip of pollen-tube ('*p.*') after fertilization, showing mucilaginous layer (*m.s.*) lining apical portion. *fil.* = filiform apparatus. 4. Two synergids ('*syn.*') treated with ammonium sulphate, showing total disappearance of filiform apparatus ('*ff.*').  
1, *Oenothera nutans* fertilized by *Ce. pyrenella*. 2-4, Hybrid '*Pycnotarpa*'. 1, 4,  $\times 1,040$ ; 2,  $\times 720$ ; 3,  $\times 1,280$ .

These reactions show that the filiform apparatus is of cellulose. Then, aqueous solution of ruthenium red was tried; the apparatus often remained entirely unstained, but sometimes it stained a pale pink or even a bright pink. This reaction indicates the occasional presence of pectic substance, which is against Habermann's result (25), i. e. he denies the association of this substance in the filiform apparatus.

*Synergid and egg-cell.* The cell wall of the synergid showed a cellulose reaction with Congo red and chlorzinc-iodide, and sometimes coexistence of pectic substance was clearly demonstrated by ruthenium red or methylene blue. As already stated, the distinct wall is differentiated around the upper two-thirds of the mature oosphere, though the remaining naked free end acquires the wall shortly after fertilization. The chemical nature of the wall is quite identical with that of the synergid. The lower naked end of the oosphere, as stated above, must be enclosed by the plasma membrane, which gave quite indifferent reactions to those reagents.

*Wall of pollen-tube.* The apical portion of the pollen-tube, which was in contact with the egg apparatus, was carefully studied. The wall stained with Congo red, and a bright pink was obtained with ruthenium red or sky blue with methylene blue. These colouring reactions show the coexistence of cellulose and pectic substance. Removal of cellulose with cuprammonia was easily done, and the residual substance fully gave the pectic reaction; but the removal of pectic substance by a weak base after the treatment with a weak solution of hydrochloric acid was entirely unsuccessful; moreover, the boiling method for the latter purpose was found quite inapplicable to the delicate section. It was often the case that, after the contents had been ejected, the wall of the apical portion of the pollen-tube was lined with some peculiar substance (Pl. VII, Figs. 8, 12, m.s.; Text-fig. VII, 1, 3, m.s.), which did not show any special colour reactions with Congo red or ruthenium red. As callose is commonly found in the upper part of the tube as the main component of the septa, treatments with corallin-soda, aniline blue, or sodium carbonate were applied to the substance in question, but it gave no callose reactions. For the control, leaves of *Ficus* containing cystoliths, stem of *Benincasa* and *Cucumis* for the callus on the sieve-plates, and pollen-tubes of *Thea japonica* possessing callose septa were fixed and treated in the same way. The callose thus treated showed the same reaction as that in the fresh condition. Thus the nature of the lining substance under discussion was left undetermined, except that, judging from the position and general appearance, it may be a kind of mucilaginous substance which seals the opening and lines the apical wall.

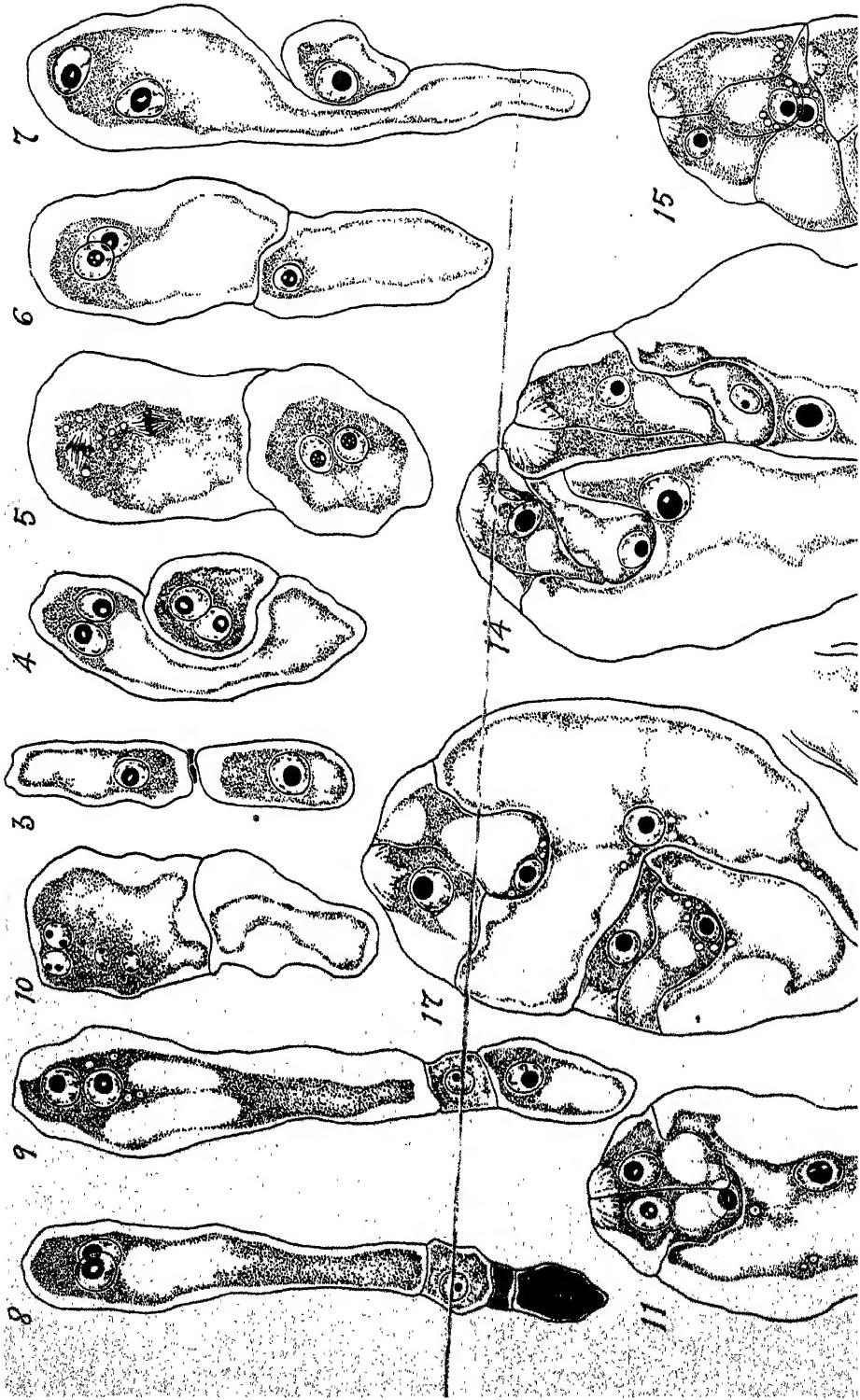
#### SELF-STERILE HYBRID BETWEEN *OE. NUTANS* AND *OE. PYCNOCARPA*.

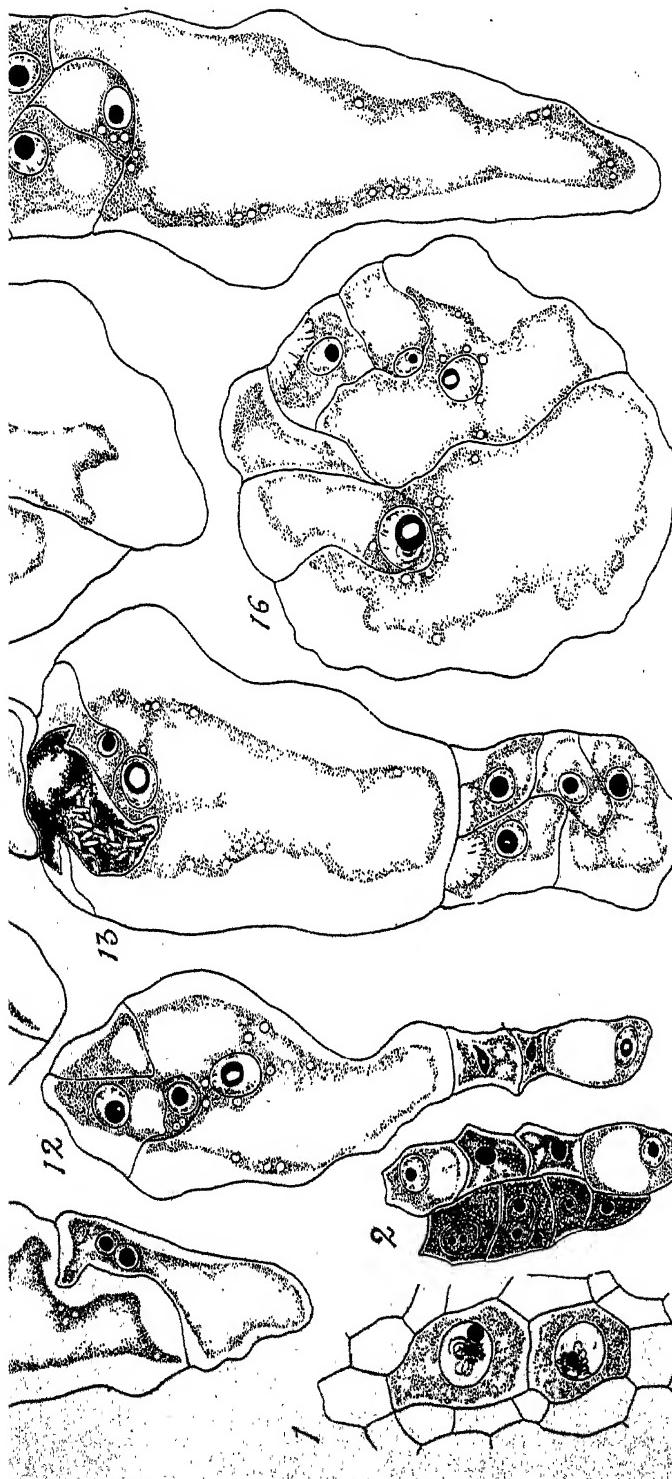
There were two forms of the hybrids between *Oenothera nutans* and *Oe. pycnocarpa*, when the materials were collected. One of the hybrids,

which was named *pycnella* by Atkinson, had flowers like those of *Oe. pycnocarpa* and was very liable to the attack of a kind of *Peronospora*, which was always parasitic only on *Oe. pycnocarpa*. This one he called a segregated hybrid, because it developed to the full expression of certain characters selected from each parent. The other, which had petals like those of *Oe. nutans* or somewhat modified by the other parent, he called *nutanella*. This was a blend, since the characters were taken from each parent, and an intermediate character of most members appeared in the hybrid. *Pycnella* was quite fertile; its pollen valid for both parents as well as for *nutanella*, *pycnella*, and itself; while *nutanella* was self-sterile, though its pollen or eggs were perfectly good when combined with those of the parent, all other hybrids, and even *Oe. Lamarckiana*. Some experiments for this self-sterility were undertaken, though it was merely preliminary. The result is that the pollen grains easily germinate, and the pollen-tubes actively penetrate the stigmatic tissues, when the self-pollination is carried on in *nutanella*. The inner morphological aspects of the gametophytes are quite normal, but the growth of the tube is so sluggish that they cannot enter the stylar tissues even in three days after pollination, when the ovules show the sign of disintegration. On the other hand, in the normal case the pollen-tube gets at the embryo sac in fifty hours; generally the ovules of *Oenothera* begin to disorganize in three days after the flowing if not visited by the pollen-tubes, and in such a case some of the embryo sacs at times show a sign of disintegration in sixty-eight hours after blooming.

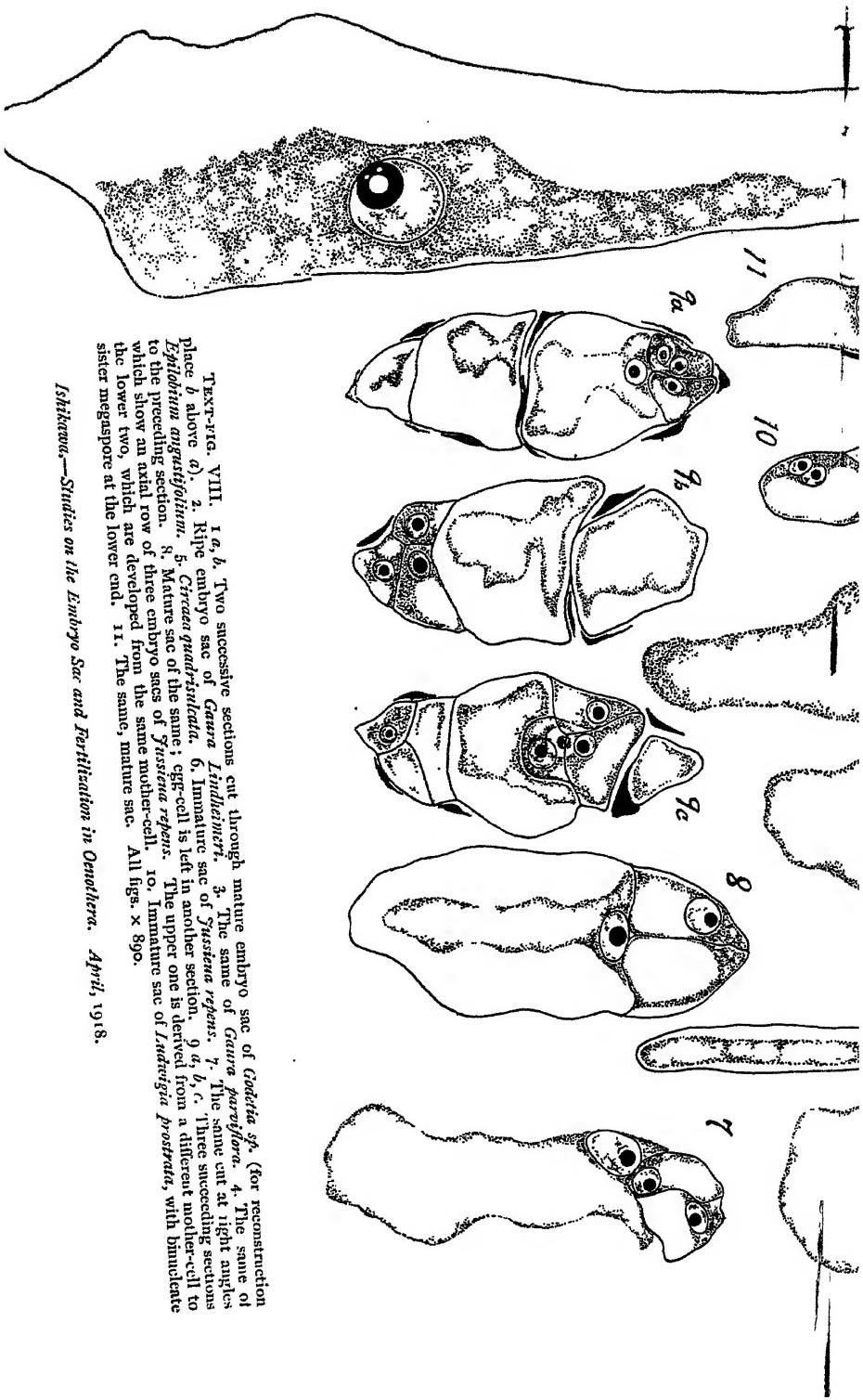
It is interesting to notice that in the hybrids, as in many cases, self-sterility is produced owing to the fact that the tube does not grow quickly enough to reach the ovule in time for fertilization. As to the cause of the feeble growth of the tube we scarcely know any explanation better than Jost's well-known hypothesis (33), namely, 'Möglich wäre auch, dass auf den Reiz des Pollenschlauches hin nicht Nährstoffe, sondern andere Stoffe im Leitgewebe sezerniert würden. Die Reaktion müsste auf fremden Pollen eintreten, und ihr Erfolg bestände in der Sekretion wachstumsreizender Stoffe.' It is also stated by Compton (10) that: 'If we compare pollination with fungal or bacterial infection it might be expected that the result would be the formation of antibodies.' 'If there be an analogy between fertilization and infection, there is also one between sterility and immunity. There seems to be two main methods by which immunity is attained. In one the host is constitutionally resistant to the attacks of the parasite, this being the more frequent method. In the other the host is over-susceptible, and the attack of the parasite produces a quantity of decomposing matter which inhibits its further growth. Both these methods are paralleled in cases of failure of fertilization.'



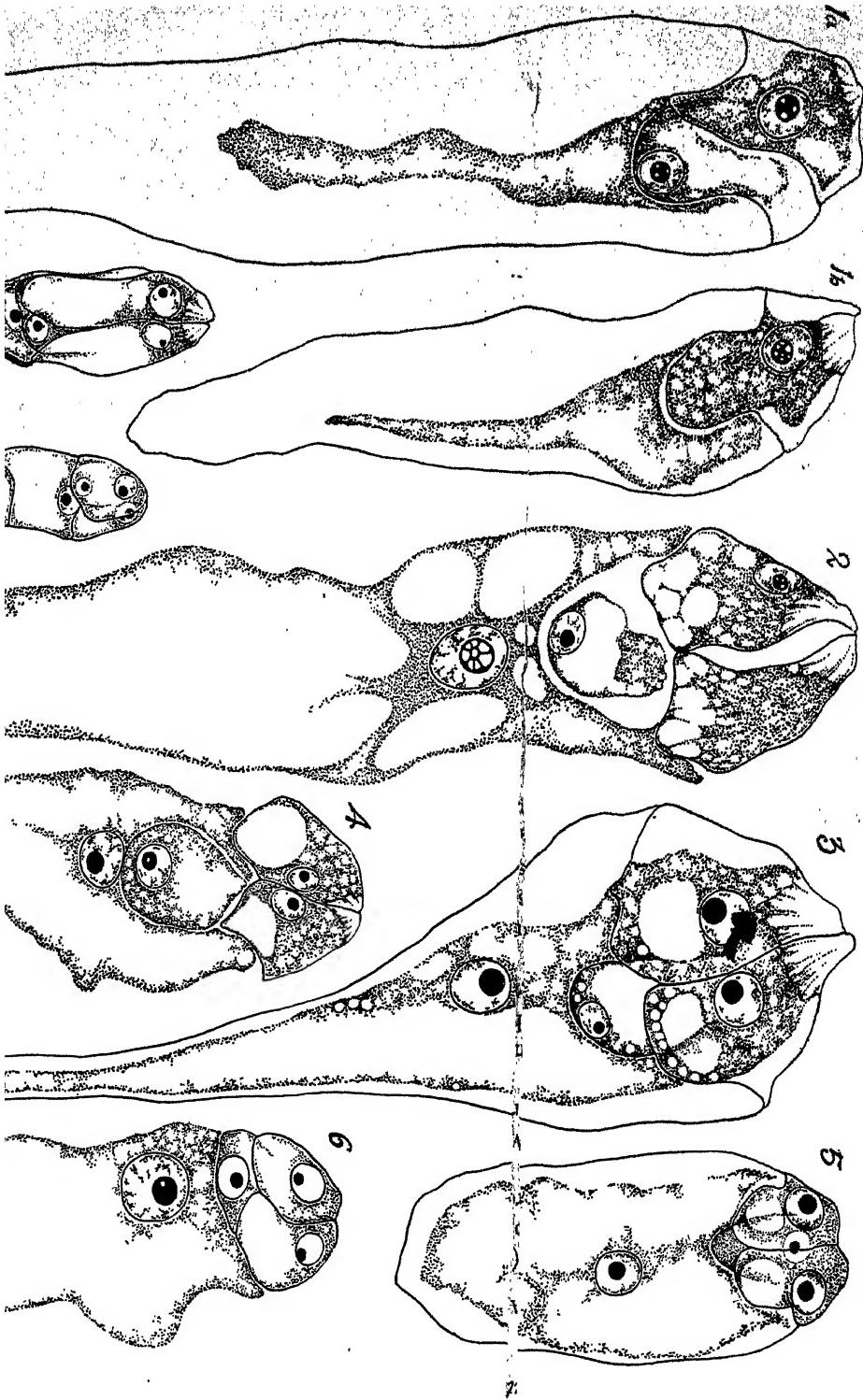




**TEXT-FIG. III.** 1. Two embryo sac mother-cells in synapsis. 2. Double axial rows of tetrads; the left one is disintegrating. 3. Microplilar and chalazal megasporangia developing. 4. Two binucleate megaspores, one of which is partly attached on the curved side of the other. 5. Upper megasporangium contains two mitotic megaspores; the lower one is binucleate. 6. Two megasporangia equally in binucleate condition; one of the nuclei is left in the adjoining section. 7. Binucleate megasporangium and the uninucleate sister one; the latter survives lying on the side of the former. 8. Upper megasporangium in binucleate condition; the middle one still survives. 9. Two excess sister megasporangia surviving. 10. Upper megasporangium contains four nuclei in teleophase; the lower one binucleate, though the nuclei are not shown in the figure. 11. Mature embryo sac with binucleate sister megasporangium, whose nuclei show a sign of disintegration. 12. Ripe embryo sac with three surviving megasporangia, two of which are going to disintegrate. 13. Two embryo sacs lying one upon the other; the upper one is fertilized. 14. Two sacs lying side by side. 15. Two sacs in axial row; the lower one is pressing the upper one. 16. Two sacs lying collaterally; part of egg apparatus is in another section. 17. One embryo sac is partly encircled by the lower half of the other; part of egg apparatus is in another section. All figs.  $\times 980$ .



TEXT-FIG. VIII. 1a, b. Two successive sections cut through mature embryo sac of *Gaudia sp.* (for reconstruction of *Gaudia parviflora*). 4. The same of *Gaudia Lindheimeri*. 3. The same of *Gaudia parviflora*. 4. The same of *Circaea quadrivalvis*. 6. Immature sac of *Tessina repens*. 7. The same cut at right angles place b above a). 2. Ripe embryo sac of *Gaudia Lindheimeri*. 5. *Circaea quadrivalvis*. 6. Immature sac of the same; egg-cell is left in another section. 8. Mature sac of the same; *Tessina repens*. The upper one is derived from a different mother-cell to the preceding section. 9a, b, c. Three succeeding sections cut at right angles to each other, the first two being derived from a different mother-cell to the preceding section. 10. Immature sac of *Ludwigia prostrata*, with binucleate eggs. 11. The same, mature sac. which show an axial row of three embryo sacs of the same mother-cell. All figs.  $\times 800$ .





## TETRANUCLEATE EMBRYO SAC.

In 1900 Chodat and Bernard (8), applying recent cytological methods, first described a tetranucleate embryo sac. This phenomenon was, however, observed by Hofmeister already in 1847 (26) and 1849 (27) in *Godetia rubicunda*, though he did not pay any special attention to the antipodal part. Geerts (21) and Modilewski (42) were the next to record, in 1909, the same cases as occurring in some plants belonging to the Onagraceae. Since then, several investigators have added some more examples to the list of such aberrant cases.

While working on *Oenothera*, the present writer also examined embryo sacs of *Gaura Lindheimeri*, *G. parviflora*, *Fussieua repens*, *Ludwigia prostrata*, and *Circaeal quadrifolia* for comparison, and found that the tetranucleate condition is of usual occurrence in these plants (Text-fig. VII, 1-12). Besides, the same has also been ascertained in *Godetia* sp. and in *Epilobium angustifolium*. Thus, the representatives of the principal genera of the Onagraceae examined, so far as the embryo sacs are concerned, are equally provided with four nuclei, as may be seen in the following table, in which all the recorded plants having tetranucleate embryo sac are given:

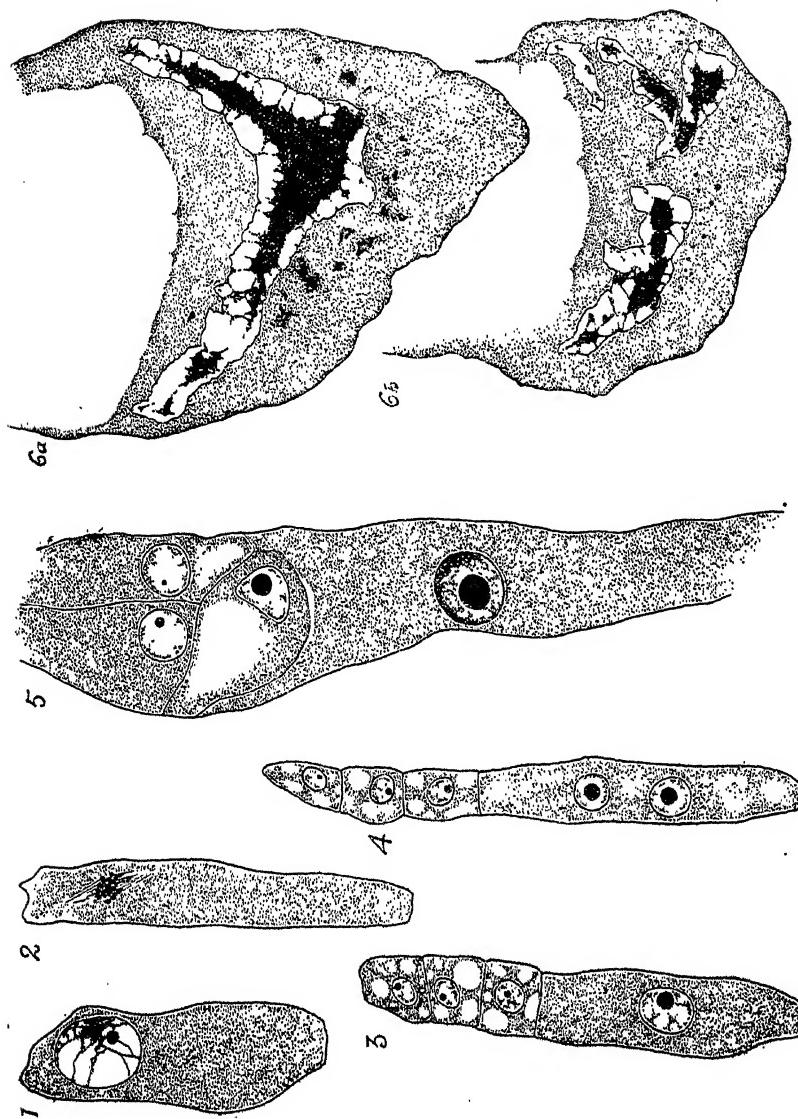
	By Smith	1910
Liliaceae :		
<i>Clintonia borealis</i>		
Orchidaceae :		
<i>Gastrodia elata</i>	Kusano	1915
<i>Cypripedium spectabile</i>	Pace	1908
<i>C. parviflorum</i>	"	"
<i>C. pubescens</i>	"	"
<i>C. candidum</i>	"	"
<i>Gyrostachys cernua</i> (not always, but in some ovules)	"	1914
<i>G. gracilis</i>	"	"
<i>Bletia Shepherdii</i>	Sharp	1912
Plumbaginaceae :		
<i>Plumbagella micrantha</i>	Dahlgren	1915
<i>Plumbago seyanica</i>	"	"
<i>P. capensis</i>	"	"
<i>P. pulchella</i>	"	"
Onagraceae :		
<i>Lopezia coronata</i>	Täckholm	1914
<i>Poasidivalia densiflora</i>	"	1915
<i>Clarkia</i> sp.	Werner	1914
<i>C. elegans</i>	Täckholm	1915
<i>C. pulchella</i>	"	"
<i>Fuchsia</i> sp.	Werner	1914
<i>F. procumbens</i>	Täckholm	1915
<i>F. coccinea</i>	"	"
<i>F. pumila</i>	"	"
<i>F. fulgens</i>	"	"
<i>F. 'Marinka'</i>	"	"
<i>F. 'Émile de Wildeman'</i> , etc.	{ Modilewski Werner Täckholm Ishikawa Täckholm	1909 1914 1915 " "
<i>Epilobium angustifolium</i>		
<i>E. hirsutum</i>		

<i>E. Doaonaei</i>	Modilewski	1909
<i>Circaea Lutetiana</i>	{ Werner	1914
<i>C. quadrisulcata</i>	Ishikawa	1915
<i>Oenothera biennis</i>	{ Modilewski	1909
	{ Werner	1914
	{ Kenner	"
<i>Oe. Lamarckiana</i>	{ Geerts	1909
	{ Werner	1914
<i>Oe. rhizocarpa</i>	"	"
<i>Oe. tetraplera</i>	"	"
<i>Oe. coccinea</i>		
<i>Oe. nutans</i>	Ishikawa	1915
<i>Oe. pycnocarpa</i>	"	"
<i>Godetia sp.</i>		
<i>G. gloria</i>	Täckholm	"
<i>G. Whittneyi</i>	"	"
<i>G. amoena</i>		
<i>Gaura Lindheimeri</i>	Ishikawa	"
<i>G. parviflora</i>	"	"
<i>Ludwigia prostrata</i>	"	"
<i>Jussiaea repens</i>		
<i>J. villosa</i>	Täckholm	"
<i>J. suffruticosa</i>	"	"
Podostemaceae :		
<i>Podostemon subulatus</i>	Magnus	1913
<i>Hydrobium olivaceum</i>	"	"
<i>Farmeria metagrioides</i>	"	"
Balanophoraceae :		
<i>Helosis guayanensis</i>	Chodat et Bernard	1900
Euphorbiaceae :		
<i>Codiaeum sp.</i>	Arnoldi	1912
<i>Ceramanthus sp.</i>	"	"
Urticaceae :		
<i>Elastostemma acuminatum</i> (in some cases)	Strasburger	1910

Thus, it may not be unreasonable to assume that all the genera of the Onagraceae are provided with a tetranucleate embryo sac except one deviated genus, *Trapa*, which has been reported by Gibelli and Ferreo (22), working on *Trapa natans*, as having a normal 8-nucleate embryo sac. According to those investigators the embryo sac of *Trapa natans* directly develops from a mother-cell just as in the case in *Lilium*. As their paper was published so long ago as 1891, these results require a re-investigation, and it is much to be desired to undertake it inasmuch as the present genus has been put into different families by different authors.<sup>1</sup>

It was therefore thought advisable to examine *Trapa natans* and its var. *incisa*. Although the material was not quite sufficient for tracing whole developmental stages, yet two essential points were clearly made out. The embryo sac mother-cell forms a tetrad due to the reduction division as shown in Text-fig. IX, 2, 3, and the chalazal megasporangium develops the embryo sac (3, 4). The mature embryo sac is very slender, slightly recurved, tapering upwards, and provided with a normal egg

<sup>1</sup> For instance, in Onagraceae by Bentham and Hooker; treated as an appendix to the Onagraceae by Eichler; included in Hydrocaryaceae by Raimann in Engler's Pflanzenfamilien; reunited into Oenotheraceae by Engler in Syll. der Pflanzenfam. (ed. 8); and treated even as an isolated member of Halorrhagidaceae by Endlicher, Lindley, Parmentier (54), &c.



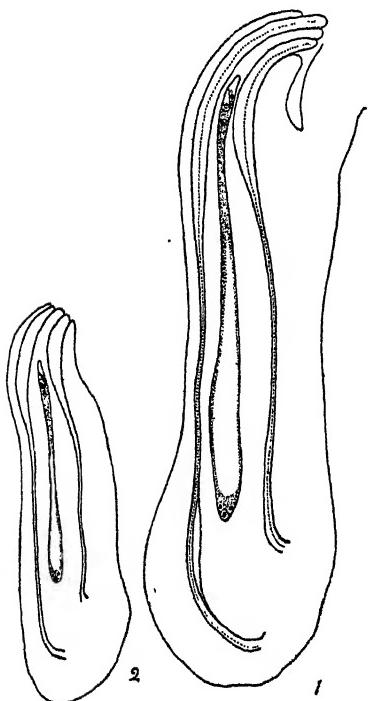
TEXT-FIG. IX. Embryo sac of *Tropaeolum*. 1. Embryo sac mother-cell; nucleus in synapsis. 2. The same; heterotype division. 3. Tetrad; lower megasporocyte growing. 4. Chalazal megasporocyte in binucleate stage. 5. Upper part of mature sac, with egg apparatus obliquely cut. 6a, b. Two succeeding sections through antipodal region; antipodal nuclei deforming. 1-4. *Tropaeolum* var. *impressum*,  $\times 600$ . 5, 6. *T. natans*,  $\times 360$ .

apparatus, secondary pole nucleus as well as antipodal nuclear group being present (Text-fig. IX, 5 and 1, 2). The antipodals always acquire no separating walls, and sooner or later each nucleus becomes chromatic, assuming an irregular shape, and finally disintegrates as shown in Text-fig. IX, 6 a, 6 b. It is often the case that two antipodal nuclei do exist instead of three, or sometimes none, probably owing to an early disintegration. This is no doubt a very interesting point, and deserves a close investigation. Thus, the result of the writer's investigation confirmed the report of Gibelli and Ferreo, except for the early stage in development. Therefore it seems not unreasonable to think that the present genus should be included in another distinct family, and it should not be regarded as representing an isolated group in the Onagraceae. This has already been suggested by Täckholm (68, 69), on account of its deviated morphological characters of the gametophyte as well as of the sporophyte.

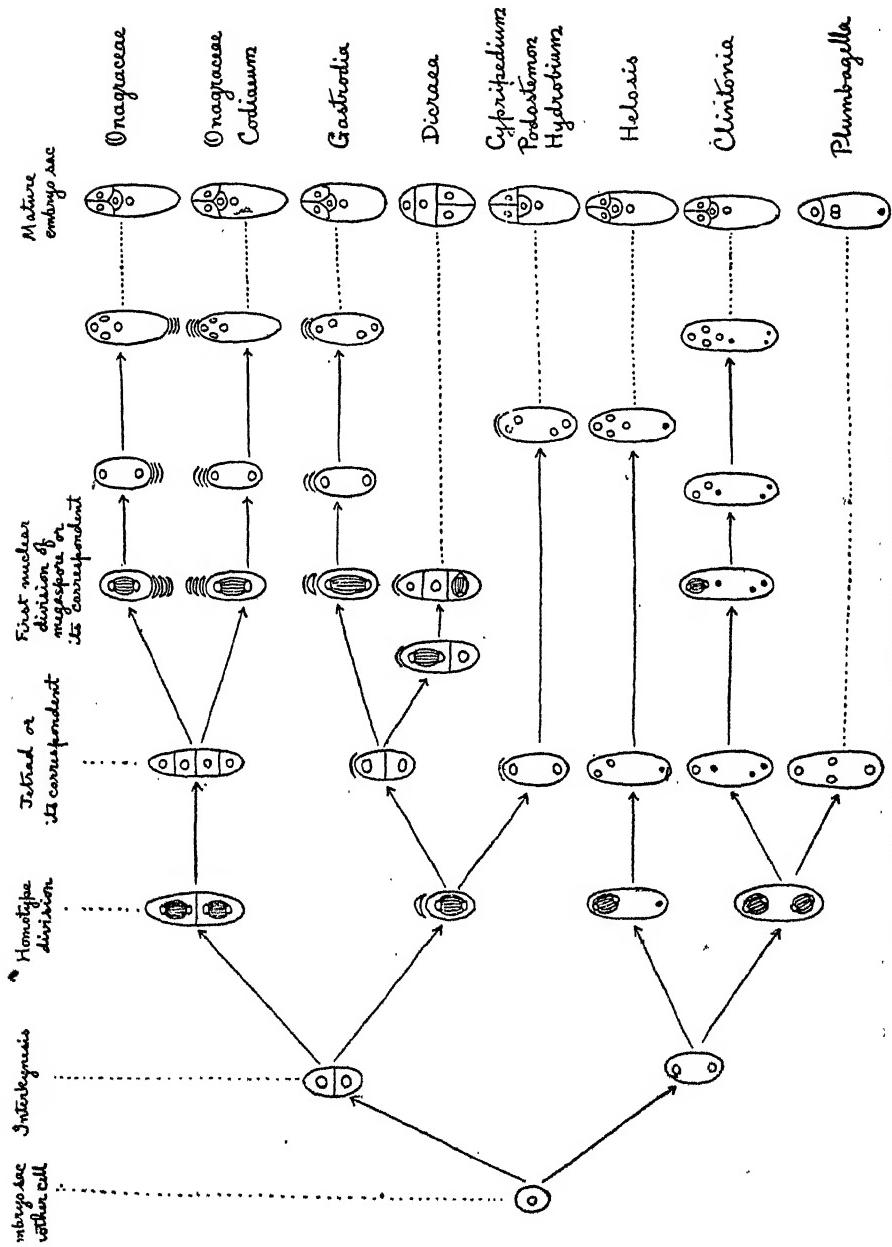
The morphological nature of the tetranucleate as well as 16-nucleate embryo sacs has been a subject of discussion by many investigators, and the prevailing opinion is to regard it as a modified form (Magnus (39), Fisher (17), &c.). The following graphical representation (Text-fig. XI), which was modified from the figures given by Fisher, Palm, and Dahlgren, would serve as a general sketch of the homology of all tetranucleate embryo sacs hitherto known to occur among the Angiosperms.

As shown in the diagram, the embryo sacs of the Onagraceae, of *Codiaceum*, of *Gastrodia*, and of *Clintonia* are of a monosporic nature, and those of *Dicraea*, of *Cypripedium*, of *Podostemon*, of *Hydrobium*, and of *Helosis* are bisporic, while in *Plumbagella* it is really tetrasporic. If in the latter case the nuclei divide once more, the sac assumes the *Lilium type*; if they divide twice, the sac acquires the type which comprises all known cases of the 16-nucleate embryo sacs.

Nearly all the tetranucleate embryo sacs do not possess antipodals, but contain a single polar in each of them. As stated above, the axis of the spindles at the second division of the nucleus of the embryo sac cell are as



TEXT-FIG. X. Sagittal section of ovule.  
x 30. 1. *Trapa natans*; 2. *Trapa natans*  
var. *incisa*.



TEXT-FIG. XI. General sketch of homology of tetranculate embryo sac.

a rule perpendicular to each other, and the one which is parallel to the main axis of the sac shares in the formation of two nuclei, of which the upper one is destined to become the egg nucleus. In *Dicraea elongata*, on the other hand, this relation is just reversed. Of the two sister nuclei brought about by the upper spindle, which is parallel to the axis of the sac, the lower one becomes the egg nucleus. The sac of *Plumbagella micrantha* is most aberrant. Although no account of the share of each nucleus in the 4-nucleate stage is given by Dahlgren (14), four nuclei give rise to one egg nucleus, two polars which unite, one antipodal which becomes disintegrated in the mature sac, and no synergid. Another curious fact regarding the behaviour of the tetranucleate sac in certain plants, such as *Cypripedium* and *Gastrodia*, is that one of the synergid nuclei fuses with the polar, thereby causing a real triple fusion at the fertilization. It is interesting to notice that the plants which have been reported as having a tetranucleate sac are all herbaceous, except *Codiaeum*. Moreover those plants shown in the following list, which are recognized as having a 16-nucleate sac, due to four successive divisions of the megasporangium nucleus, are also herbaceous. However, there is one exceptional case, which is found in *Garcinia Treubii* and *G. Kydia*. In these woody plants Treub (71) found a pentanucleate embryo sac. In *Garcinia*, when the embryo sac has attained a tetranucleate stage, one of the upper nuclei undergoes one more division, resulting in the production of a 5-nucleate sac. This is just the case with *Aglaonema*, studied by Campbell (6). The sac of *Garcinia* is, however, bisporic, while that of *Aglaonema* is tetrasporic. Other examples of pentanucleate sac have been recorded as occurring in *Lavia zeylanica* by Magnus, in *Oenone Imthurnii* and *Mourera fluviatilis* by Went (76). In these cases the sacs are bisporic, as in the case of other species in the Podostemaceae, and the nucleus of the primary embryo sac cell divides once, the upper one of the daughter nuclei undergoing two successive divisions, and the sac finally becomes pentanucleate. Besides, *Fussieua suffruticosa*, *Godetia Whitneyi*, *Fuchsia 'Marinka'*, and *F. procumbens*, have been reported by Täckholm (69) as abnormally producing two pole nuclei due to an extra-nuclear division.

As having a 16-nucleate embryo sac the following twenty-seven species of plants have so far been recorded :

Piperaceae :

	{	Campbell	1899
<i>Peperomia pellucida</i>	Johnson	1900	
<i>P. Sintensis</i>	Brown	1908	
<i>P. arifolia</i>	"	"	
<i>P. Ottomana</i>	"	"	
<i>P. hispidula</i>	Johnson	1907	
<i>P. reflexa</i>	Fisher	"	
<i>P. verticillata</i>	"	"	
<i>P. scandens</i>	"	"	
<i>P. metallica</i>	"	"	
<i>P. Fraseri</i> var. <i>resedaeiflora</i>	"	"	

<i>P. blanda</i>	Fisher	1907
<i>P. galoides</i>	"	"
<i>P. Langsdorffii</i>	"	"
Euphorbiaceae :		
<i>Euphorbia procera</i>	Modilewski	1909
<i>E. palustris</i>	"	1911
<i>E. virgata</i> (denied by Modilewski)	Dessiatoff	"
<i>Acalypha sp.</i>	Arnoldi	1912
Halorrhagidaceae :		
<i>Gunnera Hamiltonii</i>	Schnegg	1902
<i>G. chilensis</i>	{ Modilewski	1909
<i>G. macrophylla</i>	{ Ernst	1908
	{ Samuels	1912
Peneaceae :		
<i>Sarcocolla squamosa</i>	Stephens	1909
<i>S. fuscata</i>	"	"
<i>S. formosa</i>	"	"
<i>Penaea mucronata</i>	"	"
<i>P. ovata</i>	"	"
<i>Brachysiphon imbricatus</i>	"	"
Compositae :		
<i>Pyrethrum parthenifolium</i> var. <i>aureum</i>	Palm	1914

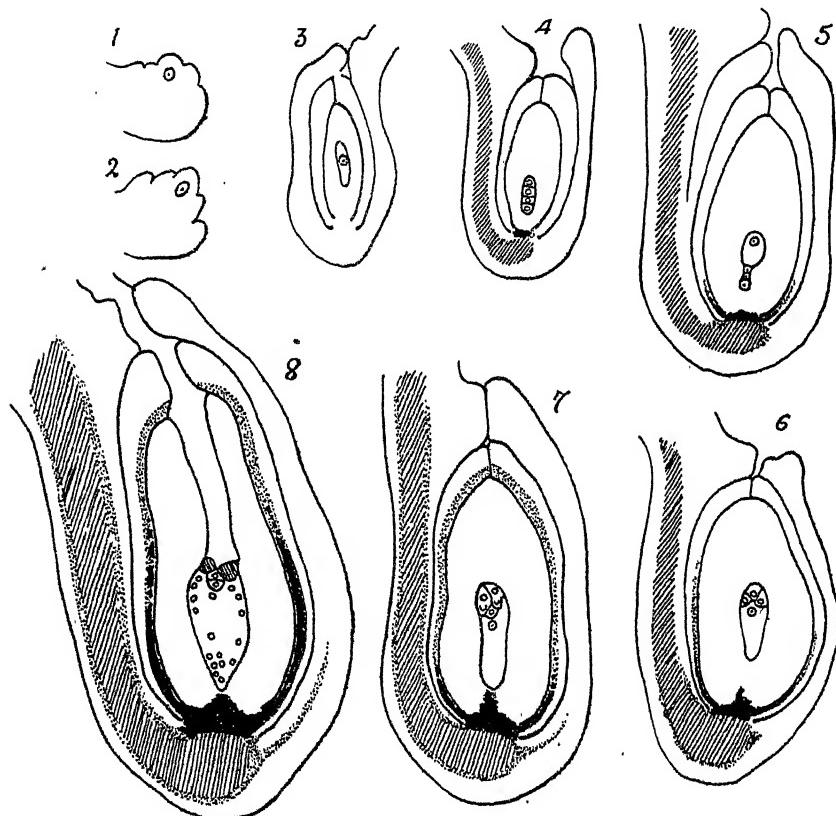
The embryo sac of *Tanacetum vulgare* studied by Palm (53) is somewhat aberrant from the 16-nucleate condition. The nucleus of the embryo sac cell, which is monosporic, divides twice; the upper two of the resulting nuclei also divide twice, while the rest of them undergo one division only, thus a 10-nucleate embryo sac results.

As already mentioned above, those owners of the aberrant embryo sacs are herbaceous. An interesting case relating to this fact has been observed by Johnson (32) and Fischer (17), who state that all the Peperomias hitherto studied are herbaceous, while another genus, *Piper*, so far as examined is found to have a 8-nucleate sac, and is a woody climber. On the other hand, Modilewski (43, 44) and Lyon state that, among fourteen species of *Euphorbia* examined, *E. procera* and *E. palustris* are 16-nucleate, while the rest possess a normal sac. Such an aberrant case of embryo sac is scarcely known to occur in the woody plants already studied of the Euphorbiaceae. Generally speaking, in the same genus or family, only herbaceous plants possess 16- or 4-nucleate sacs, while woody members have a sac of the normal type.

At any rate, tetra- and 16-nucleate embryo sacs are to be regarded as representing a derived type, probably caused by mutation and by variation, in a certain stage of phylogenetic development. Those herbaceous plants which possess such a modified gametophyte must be more evolved than others, especially than the woody members in this respect. Moreover, herbaceous plants are believed to have been derived from woody ones by a gradual decrease in amount of woody elements of vascular bundles, as held in Jeffrey's school, principally basing upon anatomical evidence. Thus the view regarding herbaceous plants as derivatives is supported by both the gametophytic and sporophytic characters.

## SOME MICROCHEMICAL OBSERVATIONS ON OVULAR TISSUES.

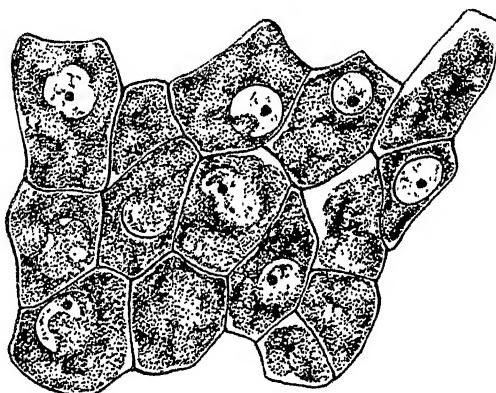
*Chromatic substance in the chalazal tissue.* In the tetrad stage of mother-cell, a cell group at the chalazal end, which borders on the base of the inner integument, becomes nearly filled with a certain liquid substance (Text-fig. XII, 4). The substance accumulates within the cytoplasm, and gradually presses the latter against the cell-wall, so much so that the cytoplasm is found as a very thin sheet lining the cell-wall and containing a much



TEXT-FIG. XII. Various stages showing distribution of chromatic substance with reference to development of ovule. Hatched part, conducting passage; darkly shaded part, tissues impregnated with chromatic substance of the first kind; dotted part, those containing substance of the second and third kind. All figs.  $\times 140$ .

compressed nucleus (Text-fig. XIII). In an advanced stage (Text-fig. XII, 5), the substance in question prevails in the chalazal cells which are situated above the end of the conducting passage, and spreads in all directions, occupying the main part of the contents of the neighbouring tissues (Text-fig. XII, 6, 7). Finally the tissue lying between the conducting passage and the embryo sac, and the basal half of the inner layer of the inner integument,

become impregnated with the substance (Text-fig. XII, 8), which is coloured black with Flemming's fluid or beautifully stained with safranin, malachite green, and iron-alum-haematoxylin in the fixed material. According to Werner (73) those cells which contain the chromatic substance are destitute of nucleus due to gradual disintegration. In the material examined by the writer, however, they possess sound nuclei (Text-fig. XIII) even at the time of endosperm formation; they are very easily seen if treated with yellow prussiate and chloride of iron. Before the first appearance of such impregnation, liquid substance of another kind, which is stained with light green in the fixed material and gives a tannin reaction with chloride of iron in the fresh condition, appears in the lowermost cells of the inner integument and in those of the neighbouring region at the terminal end of the conducting tissue (Text-fig. XII, 4).



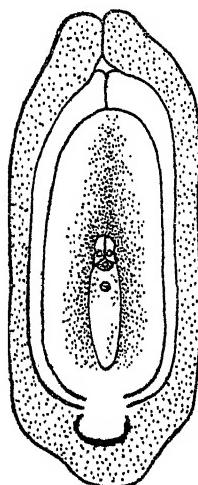
TEXT-FIG. XIII. Portion of chalazal tissue containing chromatic substance.  $\times 900$ .

Then it spreads to the inner cell layer of the inner integument, but sooner or later it becomes replaced by the one first mentioned, perhaps owing to a chemical change, except that in the upper half of the integument, which remains unchanged. Moreover, in a mature ovule a cylindrical layer of cells surrounding the conducting passage and a sheet of cells in the basal part of the outer integument are also filled with granular substance, which, in the fixed material, stains also with light green. Though this chromatic substance of the third kind at least may contain tannic substance, the chemical nature is left undecided, for the test tried was found unsuccessful to determine it. As the cells which contain any of those three kinds of the chromatic substance stain light black colour with Sudan III in the fixed material, it was found advisable to study the ovules in fresh condition. But having no material of *Oe. nutans* or *Oe. pycnocarpa* at hand, *Oe. biennis* var. *canescens* and *Oe. odorata* were used, which are nearly the same as far as the internal structure are concerned. The result is as follows:

Reagent.	Chromatic substance of the first kind accumulated in the chalazal region.	Chromatic substance of the second kind.
$\alpha$ -naphthol + $H_2SO_4$	—	—
Sudan III	—	—
Osmic acid	black	black
Millon's reagent	black	black
Eosin	—	slightly stainable
$FeCl_3$	—	black
$HNO_3$	yellow	—
$NaOH$ after $HNO_3$	brownish yellow	—
Iodine	—	—
Alcohol	insoluble	soluble
Ether	soluble	

Judging from the results tabulated above, the substance of the first kind in question must be some ultimate substance which does not share in the nutritive process, while the second kind of the substance under discussion is really a solution of tannin without question.

*Starch grains in nucellar tissue.* Iodine solution was employed for detecting starch grains both in fixed and fresh materials. In the tetrad stage of the mother-cell, starch grains accumulate in the nucellar tissue, especially densely surrounding the gametophyte as well as along the future passage of the pollen-tube, where they are found in large granules; perhaps some of them become glucose in due time, which serves to attract the pollen-tube. The grains are also deposited in the chalazal tissue neighbouring the terminal portion of the conducting passage. No further change in the distribution takes place up to the time of embryo formation (Text-fig. XIV), when they gradually disappear. Starch grains are totally absent in the inner integument, while they accumulate more or less in the basal part of the outer integument, and as the embryo begins to develop they spread over the latter.



TEXT-FIG. XIV. Longitudinal section of ovule showing distribution of starch grains by means of dots.

As is often the case in many plants, the ovule of *Oenothera* has an inner integument, whose internal cell-wall facing the nucellus is already fully cuticularized in the mother-cell stage of the gametophyte, as demonstrated with Sudan III. Consequently in the growing ovular tissue, nutritive communication of the nucellus with the raphae is restricted only to the chalazal portion, where the chromatic substance in question makes an early appearance and forms so-called hypostase of Van Tieghem.

Thus, the case would be very easily explained if we assumed the chalazal chromatic substance as a certain nutritive substance which is

produced in correlation with accumulation of starch grains in the nucellar tissue. But the result of microchemical observations denies any nutritive substance existing there, and, on the contrary, it suggests something like ultimate products.

The writer therefore examined several species of other genera of the Onagraceae with a view to finding a clue to solve the question. Unfortunately, the result of the investigation did not fully answer the expectation, but a close relation existing between the hypostase and starch grains in nucellus was found, which is shown in the following table:

<i>Material.</i>	<i>Starch grains in the nucellus.</i>	<i>Hypostase, i.e. the tissue containing chromatic substance in the chalazal region.</i>	<i>Cuticularization of the walls of the inner integment.</i>
<i>Oenothera nutans, pycnocarpa, biennis</i> var. <i>canescens</i> , and <i>odorata</i>	many	+	+
<i>Gaura parviflora</i>	many	+	+
<i>G. Lindheimeri</i>	many	+	+
<i>Circaeæ quadrivalvisculata</i>	many	+	+
<i>Ludwigia prostrata</i>	not so many	-	+
<i>Fussieua repens</i>	few	-	+
<i>Epilobium angustifolium</i>	very few	-	+
<i>Godetia</i> sp.	none	-	+
<i>Fuchsia macrostemma</i>	none	-	+

From this table it can be seen clearly that the deposition of numerous starch grains generally occurs when the hypostase is present, and that the chromatic substance interferes with nutritive communication between the nucellus and conducting passage.

Lastly, the question may be discussed from the phylogenetic point of view. As to the affinity among the genera of the Onagraceae, Parmentier's taxonomical and anatomical investigation (54) led to the conclusion that the most primitive type was found in the group to which *Ludwigia* and *Fussieua* belong, and that from this group *Oenothera* was originated and gave rise to other genera. Accepting his conclusion, and reflecting on the result of the present investigation, we can assume that the primitive types scarcely possess hypostase, while in certain derived genera, such as *Oenothera* and *Gaura*, the tissue in question is present. It is curious, however, that *Godetia*, a genus closely related to *Oenothera*, is entirely destitute of this tissue. In *Epilobium*, which is a direct derivative of *Oenothera*, this tissue disappears secondarily, and the same is also the case with *Fuchsia*, a genus allied both to *Oenothera* and *Ludwigia*. On the other hand, it still exists in an isolated genus, *Circaeæ*.

It may therefore be concluded that the presence or absence of hypostase and starch grains has no relation with the tetranucleate condition of the embryo sac which is a diagnostic character of the Onagraceae.

## ECOLOGICAL CONSIDERATION OF TETRANUCLEATE EMBRYO SAC.

Of the papers dealing with ecological consideration of the tetranucleate embryo sac, those by Magnus (39) and by Kusano (36) seem to be the only ones in which the authors try to explain the origin of the modified embryo sac in detail. According to the former investigator, the feeble reproductive part of the Podostemaceae lifts itself above water at the flowering time, and becomes exposed to the direct sunlight of the Tropics. It is therefore subjected to a xerophytic condition under which, and within only a few days' time, the gametophytic part of the life cycle should be completed. Thus, an abbreviation of certain processes in the gametophyte and some amount of modification in the nucellar tissues are necessarily brought about by pressing need for a rapid growth. Kusano states that in *Gastrodia*, as the reproductive organs develop at the expense of the food material stored in the subterranean tuber alone, the nutritive substance must be economically distributed to the organs, especially because of the great rapidity of growth of the flower shoot, gametophyte, and the fruit. Thus, he attributes the origin of the abbreviation of the sac to the extreme restriction of the food and time, and concludes that the physiological adaptation in the gametophyte has brought forth morphological adaptation. And, according to him, the embryo is fed for a time with nourishment in the sporophytic tissue, as is the case with the Podostemaceae. However, in the case of *Oenothera* and other genera of the Onagraceae there is no such pressing ecological condition which can explain the occurrence of the aberrant embryo sac. Nor does any morphological or physiological deviation happen in the ovular tissues, although a curious relation exists between the hypostase and starch grains, which apparently has nothing to do with the tetranucleate gametophyte and development of the embryo. Thus, the writer is led to the conclusion that the tetranucleate condition of the gametophyte of the Onagraceae was caused by mutation, but the subsequent physiological counterbalance was not so markedly disturbed, that it became fixed without any special modification of the surrounding tissues.

## ADDITIONAL NOTE.

Among the tetranucleate embryo sacs of the Onagraceae examined by the writer, those of *Godetia* are the largest, while those of *Ludwigia* are the smallest. The sac of *Gaura* comes next to that of *Godetia*, then follow those of *Epilobium*, *Oenothera*, *Circaeja*, and *Fussieua*, as shown in Text-fig. VIII, 1-12. In Text-fig. VIII, 9 a, 9 b, 9 c, three successive sections of three developed embryo sacs are shown, which especially are worth mentioning; if they are reconstructed, three mature sacs, which are arranged in an axial row, are obtained. Judging from the remnants of the tapetal cells, the

uppermost embryo sac is derived from a different mother-cell, while the middle and lowest sacs have a common origin from another one.

#### SUMMARY.

1. The embryo sac arises from either micropylar or chalazal one of the tetrad; often both of them simultaneously develop into complete embryo sacs.
2. The embryo sac is tetranucleate, lacking the antipodals and one of the pole nuclei.
3. The pollen-tube enters the synergid through the filiform apparatus and the mixed plasma flows out through the synergid and spreads over the oosphere.
4. The plasma of the pollen grain is impregnated with an immense number of minute fusiform starch grains, which migrate through the pollen-tube, enter the synergid, and finally become entirely consumed.
5. The male nucleus is enclosed in a distinct plasma sheath, until it reaches the oosphere.
6. One of the male nuclei fuses with the pole nucleus, and gives rise to the endosperm nucleus with diploid number of chromosomes.
7. Synergids and upper two-thirds of the oosphere have a distinct cellulose membrane which sometimes contains also pectic substance. The lower part of the egg-cell acquires a cellulose membrane after fertilization.
8. Self-sterility of some hybrids is due to a feeble growth of the pollen-tube.
9. Besides *Oenothera*, *Ludwigia*, *Gaura*, *Godetia*, and *Circaea* also produce tetranucleate embryo sacs, which is a diagnostic character of the Onagraceae. On the other hand, *Trapa*, which has a normal 8-nucleate embryo sac, would better be separated from the Onagraceae.
10. The chalazal tissue is nearly occupied by some chromatic substance which appears to be some ultimate substance. Many starch grains accumulate in the young nucellar tissue before the chromatic substance makes an appearance.
11. In other genera examined of the Onagraceae, the nucellar tissue is nearly or entirely devoid of deposition of starch grains, correlated with the absence of the chromatic substance.
12. Tetranucleate condition in the Onagraceae may have been produced by mutation, but not by adaptation. No relation exists between the tetranucleate condition and the presence of the chromatic substance.
13. The species with 4- or 16-nucleate embryo sacs are mostly herbaceous.

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#### EXPLANATION OF FIGURES ON PLATE VII.

Illustrating Mr. Ishikawa's paper on the Embryo Sac and Fertilization in *Oenothera*.

Figs. 1, 6, 8-10, 12, 17, hybrid 'pycnelia'; 2-5, 7, *Oenothera nutans*; 11, 13-16, 18, *Oe. nutans* fertilized by *Oe. pycnocarpa*. 13-15  $\times$  900; 1-9, 13-16, 17, 18  $\times$  1,300.

Lettering used is as under:

e.c., egg-cell; e.d., endosperm nucleus; e.n., egg nucleus; fil., filiform apparatus; m.n., male nucleus; m.s., mucilaginous substance; p., pollen-tube; p.f., plasma flow from synergid; p.n., pole nucleus; p.s., plasma sheath of male nucleus; rs., residue in pollen-tube; syn., sound synergid; syn., attacked synergid; s.n., nucleus of synergid; v., vegetative nucleus.

Fig. 1. Mature pollen grain containing degenerated vegetative nucleus (*v.*) in centre and elliptical generative one (*g.*) lying in peripheral portion. Plasma is impregnated with immense numbers of hyaline fusiform starch grains.

Fig. 2. Pollen-tube in stylar conducting tissue, showing disorganized vegetative nucleus (*v.*), two male nuclei (*m.n.*) in telophase with plasma sheath, and migrating starch grains.

Fig. 3. The same, showing a disintegrating vegetative nucleus (*v.*).

Figs. 4-6. Tips of pollen-tubes just lying upon embryo sacs, each of them containing two male nuclei embedded in plasma sheath, no vegetative nucleus.

Fig. 7. The same, typical lenticular male cell is shown.

Fig. 8. Two tubes (*A.*) on the sac, one of which made the way between two filiform apparatus (*fil.*). Apical end of the tube is closed by mucilaginous substance (*m.s.*). Synergids (*syn.1*) are filled with starch grains which originated in the pollen grain.

Fig. 9. *a, b.* Two successive sections of an egg apparatus cut longitudinally; *a* is to overlap *b* in the actual state of affairs. Upper synergid (*syn.1*) has been attacked by tube (*p.*), contents commencing to flow out over oosphere (*e.c.*) through rents (*r., r., r.*).

Fig. 10. Upper part of embryo sac with six endosperm nuclei, two of which (*e.n.*) are shown in both sides of egg-cell (*e.c.*).

Fig. 11. Egg apparatus with two synergids attacked (*syn.1*) by tube (*p.*).

Fig. 12. The same, but one synergid attacked.

Fig. 13. Oosphere cut slantingly at the lower end; two male nuclei (*m.n.*) enclosed in plasma sheaths (*p.s.*) lying in plasma flow poured out from synergid.

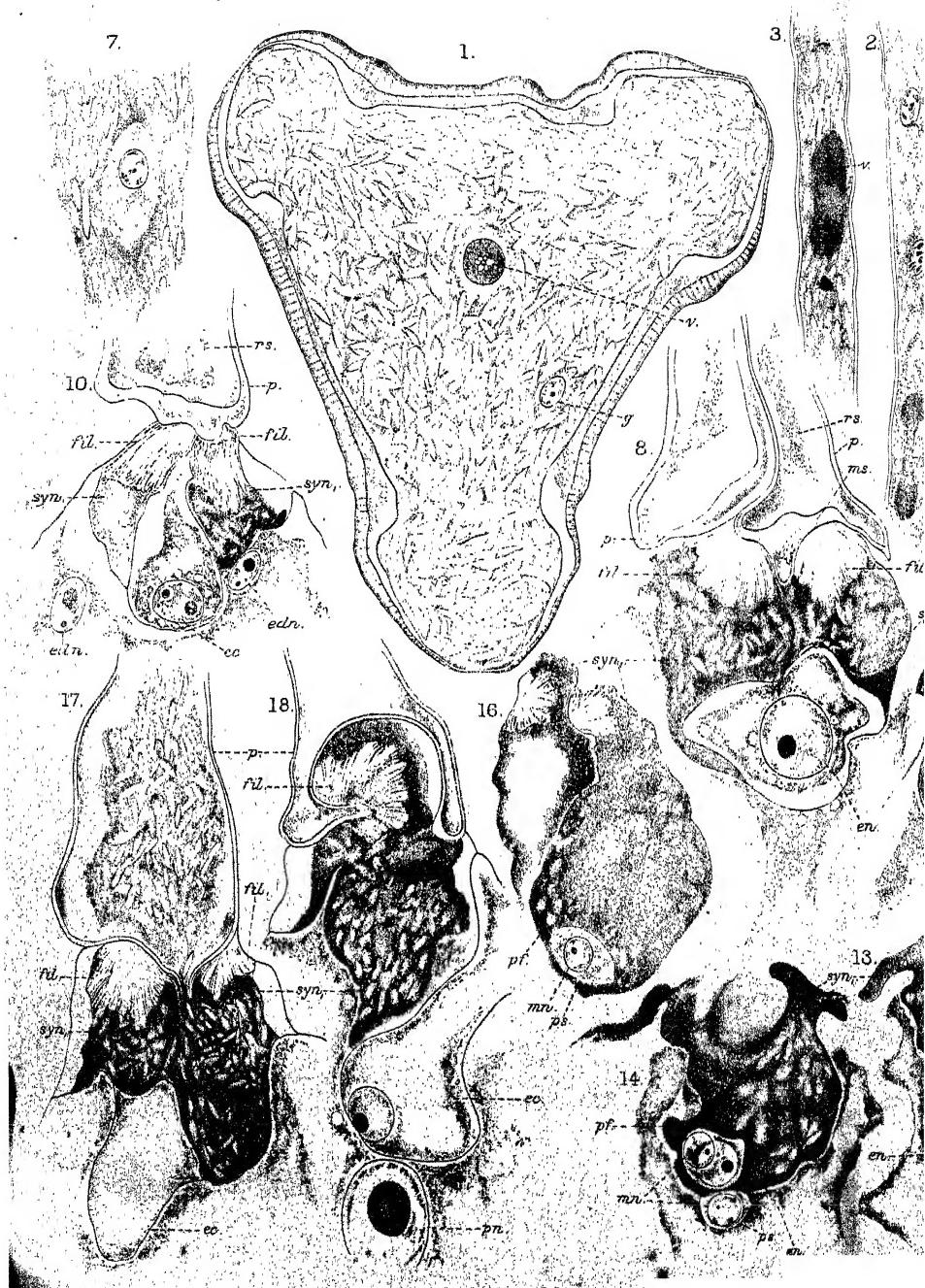
Fig. 14. Two male cells (*m.n.* and *p.s.*) in plasma flow (*p.f.*), through the upper one of which egg nucleus (*e.n.*) is visible.

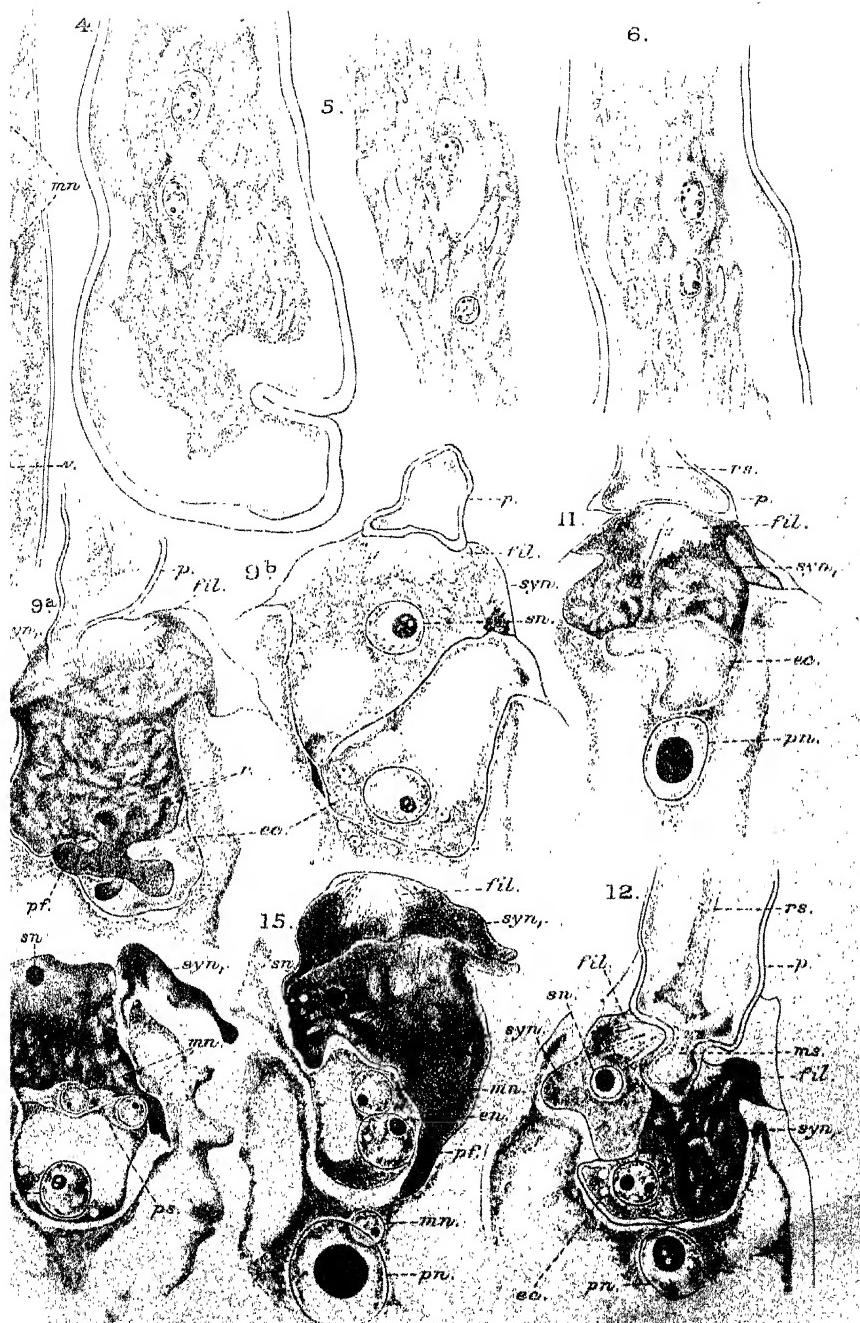
Fig. 15. Two male nuclei (*m.n.*) are just in contact with female elements (*e.n., p.n.*).

Fig. 16. Plasma flow upon oosphere. One male cell is shown.

Fig. 17. Tube end bursting, starch grains flowing into synergid.

Fig. 18. Two branchlets of pollen-tube grasping tip of synergid, filiform apparatus (*fil.*) turned over, portion of mixed plasma contents flowing over the latter.







## Studies on some East Indian Hepaticae.

BY

D. H. CAMPBELL.

With Plates VIII and IX and ten Figures in the Text.

### DUMORTIERA.

THE genus *Dumortiera* is common throughout the humid tropics of both hemispheres, and extends into the warm temperate regions as well. Thus *D. hirsuta* occurs in the British Isles, Japan, and the South-Eastern United States.

The anatomy of the thallus has been pretty completely investigated and will not be dwelt upon at length.<sup>1, 2</sup> Very little, however, has been done upon the development of the reproductive organs and sporophyte.

Collections have been made by the writer in various parts of the Indo-Malayan region. These were mostly of the widely distributed species, *D. trichoccephala*, Hook., but the much more local species, *D. velutina*, Schiffn., was also collected, as well as what appears to be an undescribed species from Sarawak in Borneo.

From a study of these collections it was possible to secure a fairly satisfactory series of preparations showing the most important points in the development of the sexual organs and sporophyte.

Stephani<sup>3</sup> recognizes but three species of the genus, of which *D. trichoccephala* is widely distributed throughout the Indo-Malayan region and Oceanica, while *D. hirsuta* (Sw.), R. Bl., is widespread in both hemispheres. The very distinct *D. velutina*, Schiffn., is known only from Java and Sumatra. Immature specimens of *Dumortiera*, collected by the writer in Hawaii during the past summer, may possibly belong to this species.

In 1892 the writer collected in Hawaii sterile specimens of a *Dumortiera* which was identified by the late Professor L. M. Underwood as *D. trichoccephala*.<sup>3</sup> The thallus in this form is quite destitute of even the rudiments of air-chambers, thus differing from all the other species that have been

<sup>1</sup> Leitgeb : Untersuchungen über die Lebermoose, vi. Graz, 1881.

Ernst, A. : Untersuchungen über Entwicklung, Bau und Verteilung der Inflorescenzen von *Dumortiera*. Ann. Jardin Bot. Buitenzorg, 2<sup>o</sup> sér., vii, 1908, pp. 153-223.

<sup>2</sup> Stephani, F. : Species Hepaticarum. Bull. L'Herbier Boissier, vii, 1899, pp. 222-5.

<sup>3</sup> See Campbell : Mosses and Ferns, 2nd edition, p. 49.

investigated. Fertile material of this form, which is very abundant in many parts of Hawaii, was collected by the writer during the past summer, and it is doubtful whether it can properly be referred to *D. trichocephala*.

All of the species of *Dumortiera* are characterized by a more or less complete suppression of the air-chambers which are so conspicuous a feature of the thallus of the typical Marchantiaceac. In the Hawaiian form referred to above, no trace of the air-chambers could be detected, even in the youngest portions of the thallus; but in the other species more or less conspicuous remains of the air-chambers are visible. Ernst<sup>1</sup> has studied carefully these air-chambers in *D. trichocephala* and *D. velutina*, and the writer can confirm his results from an examination of material from the same localities in Java where Ernst collected his specimens.

In *D. velutina* not only are the air-chambers very evident near the apex of the thallus, but the green tissue found in the air-chambers of the typical Marchantiaceae is represented by crowded papillate cells which completely cover the surface of the thallus and give it a characteristic velvety texture, very different from the greasy-looking, olivaceous, nearly smooth surface in *D. trichocephala*. In the latter the papillate cells are either completely absent or very sparingly scattered over the surface.

In all species the epidermis is completely wanting except in the youngest parts of the thallus; but the lateral walls of the air-chambers may persist more or less completely so as to form an irregular reticulation on the surface comparable to that present in the typical Marchantiaceae (see Pl. VIII, Fig. 3).

The development of the air-chambers is, to some extent at least, associated with the environment.<sup>2</sup> When the conditions are comparatively dry the air-chambers are better developed than in a very wet situation, and excessive moisture may cause a complete suppression of the air-chambers. Of the species considered in the present paper, *D. calcicola*, which grows under relatively dry conditions, has the air-chambers comparatively well developed.

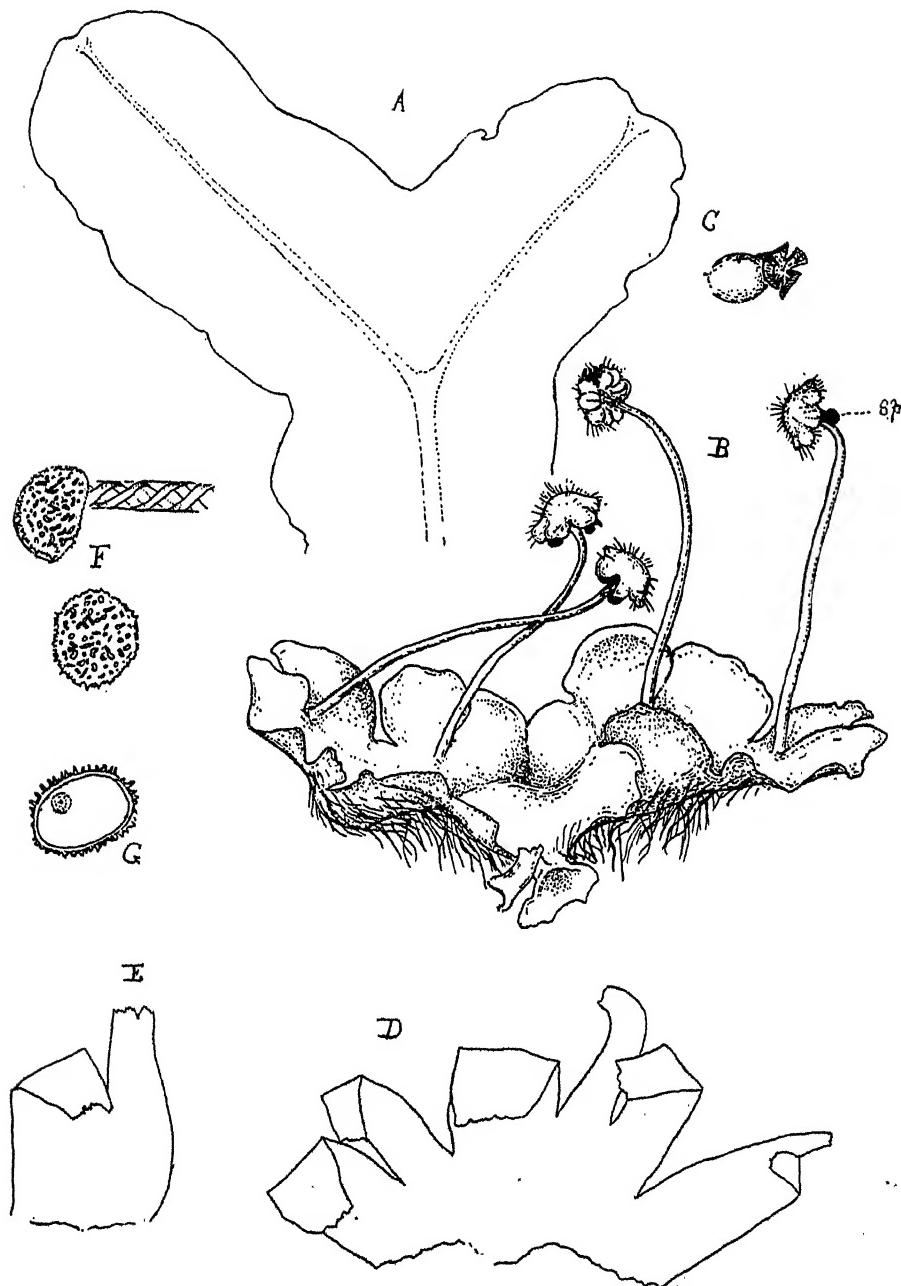
That moisture is not the only factor concerned, however, is probable. During the past summer the writer collected two species of *Dumortiera* in Hawaii not infrequently growing together, one of which had conspicuous reticulations on the surface of the thallus, while the other showed no trace of air-chambers.

#### *The Reproductive Organs.*

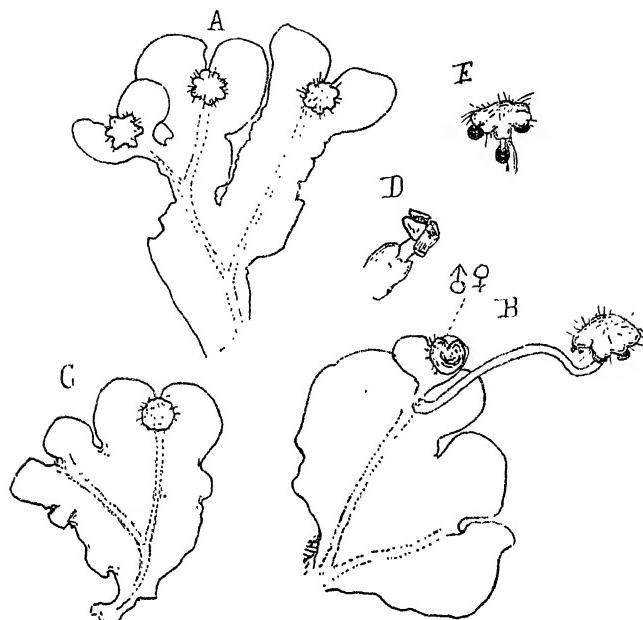
As in other Marchantiaceae, the sex-organs in *Dumortiera* are borne on the characteristic receptacles. The male receptacle (Pl. VIII, Fig. 6, II; Text-fig. 2, C) is a nearly flat disc, borne upon a very short pedicel. The female receptacle, or carpocephalum, is more or less conspicuously convex, and at

<sup>1</sup> Loc. cit.

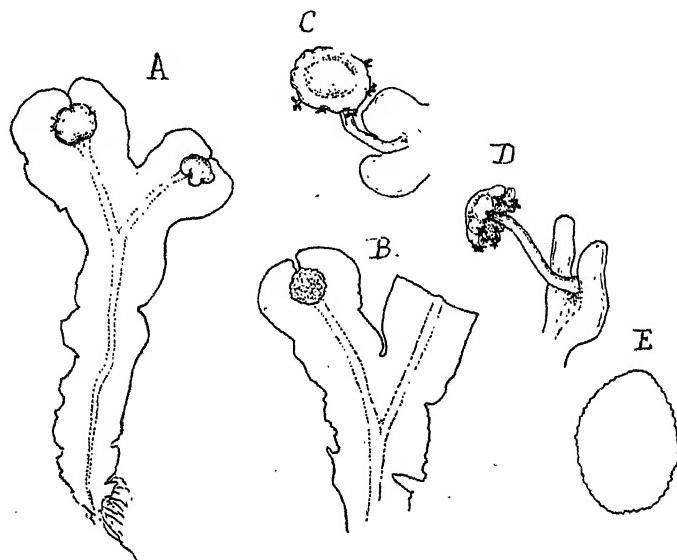
<sup>2</sup> Coker, W. C.: Selected Notes, II. Bot. Gaz., xxxvi, 1903, pp. 225-30.



TEXT-FIG. 1. *Dumortiera trichocephala*, Hook. A. Large sterile specimen from Luzon, Philippine Islands.  $\times 1\frac{1}{2}$ . B. Fertile plant from Tjibodas, Java.  $\times 1\frac{1}{2}$ . C. Open sporogonium.  $\times 3\frac{1}{2}$ . D, E. Open capsule, showing the deeply divided valves.  $\times 20$ . F. Ripe spores and part of an elater.  $\times 575$ . G. Section of ripe spore.  $\times 575$ .



TEXT-FIG. 2. *Dumortiera trichocephala* (Taiping Hills, Federated Malay States). A. Female plant with young carpocephala.  $\times 1\frac{1}{2}$ . B. Individual with a ripe carpocephalum and an androgynous receptacle ( $\delta\varphi$ ).  $\times 1\frac{1}{2}$ . C. A male plant.  $\times 1\frac{1}{2}$ . D. Open sporogonium.  $\times 3\frac{1}{2}$ . E. Carpocephalum, with ripe sporogonia.  $\times 2$ .



TEXT-FIG. 3. *Dumortiera velutina*, Schiff. A. Female plant with young carpocephala.  $\times 1\frac{1}{2}$ . B. Male plant.  $\times 1\frac{1}{2}$ . C, D. Mature carpocephala.  $\times 2$ . E. Ripe spore.  $\times 575$ .

maturity there is usually a very much elongated pedicel. This may occasionally in *D. trichocephala* reach a length of 7 cm. or more (Text-figs. 1, 2, 3).

It is evident that both male and female receptacles are of the 'composite' type; i. e. they are branch systems resulting from the rapid and repeated dichotomy of the apex of the shoot which terminates in the receptacle. To judge from a somewhat casual study of the young receptacles, they do not differ in any way from those of various other Marchantiaceae that have been described (see Cavers,<sup>1</sup> Leitgeb,<sup>2</sup> Campbell<sup>3</sup>).

In *D. trichocephala*, which is dioecious as a rule, occasionally specimens are met having both male and female receptacles. In such cases the male receptacle is first formed, and later, upon an adventitious shoot developed from the ventral side of the thallus, near the apex, a carpocephalum may be developed. In this species, as Ernst first showed,<sup>4</sup> androgynous receptacles are not infrequently met with (see Text-fig. 2, B).

*D. velutina*, according to Schiffner,<sup>5</sup> is always dioecious, and the writer has not observed monoecious individuals in this species, nor any androgynous receptacles.

The antheridial receptacles are flattened discs with a more or less marked central depression (Text-fig. 4, A). The pedicel is very short. On the lower side of the receptacle are formed narrow scales much like those near the apex of the sterile branches. The antheridia are developed in acropetal succession from the several marginal growing points of the receptacle, arising from the dichotomy of the original shoot-apex. There is considerable displacement of the older antheridia, so that they are not arranged in such definite radiating rows as is the case in *Marchantia*.

The development of the antheridium is much as in other Marchantiaceae.<sup>6</sup> The somewhat elongated papillate mother-cell, after cutting off a basal cell, as in *Marchantia*, is divided first by transverse walls into three or four superimposed cells. Each of these (except the terminal one) then divides, by intersecting vertical walls, into four, and these, by the formation of periclinals, are separated into the inner spermatogenic tissue, and the outer layer of sterile cells constituting the antheridium wall (Text-fig. 4, B-E).

The stalk-cell undergoes numerous transverse divisions, with an occasional longitudinal division in the upper cells, and a slender stalk is

<sup>1</sup> Cavers, F.: The Inter-relationships of the Bryophyta. New Phytologist, Reprint, No. 4, Cambridge, 1911.

<sup>2</sup> Leitgeb: loc. cit.

<sup>3</sup> Campbell: loc. cit.

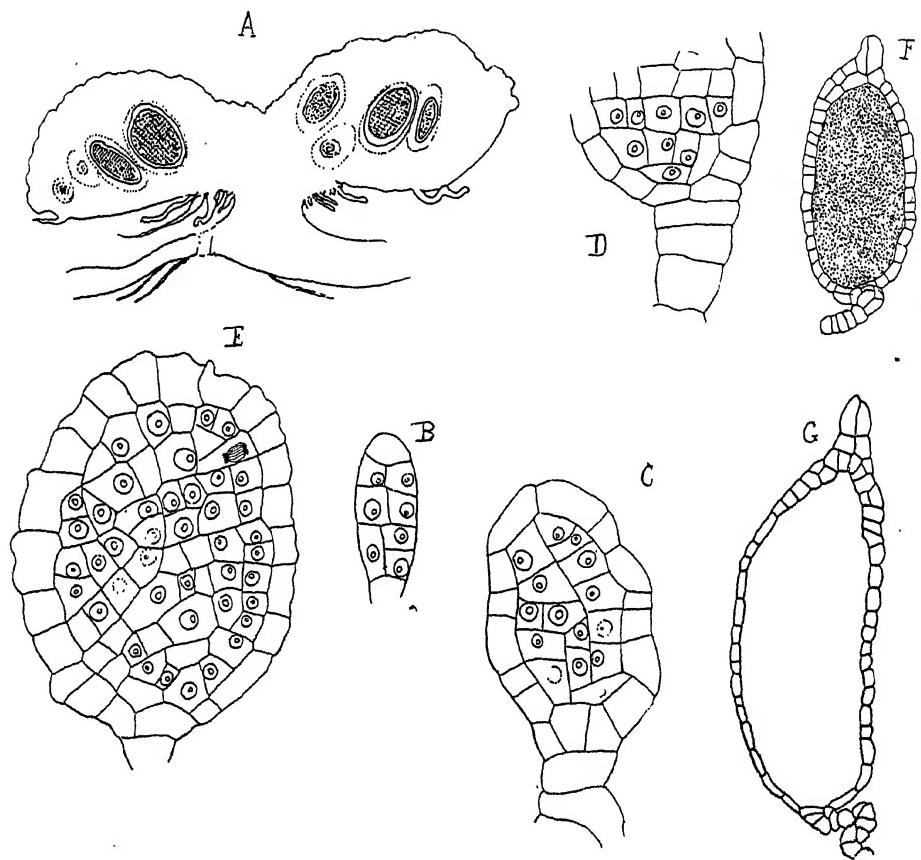
<sup>4</sup> Ernst: Über androgyne Inflorescenzen bei *Dumortiera*. Ber. Deutsch. Bot. Gesellsch., xxv, 1906, pp. 455-64.

<sup>5</sup> Schiffner: Die Hepaticae der Flora von Buitenzorg, xxvi. Leiden, 1900.

<sup>6</sup> Strasburger: Die Geschlechtsorgane und die Befruchtung bei *Marchantia polymorpha*. Pringsh. Jahrb., v, 1866-7, p. 297. Campbell: loc. cit., pp. 49-53.

thus formed (Text-fig. 4, F, G). This elongated stalk is unusual among the Marchantiaceae, but has been also noted in *Targionia*.<sup>1</sup>

The apex of the antheridium becomes elongated and forms a conspicuous beak (Text-fig. 4 F, G).



TEXT-FIG. 4. A. Section of an antheridial receptacle of *D. trichocephala*.  $\times 35$ . B-E. Young antheridia of *D. trichocephala*.  $\times 350$ . F. Ripe antheridium.  $\times 65$ . G. Antheridium of *D. velutina*.  $\times 65$ .

The development of the spermatozoids was not investigated in detail, but the last division of the spermatocytes is not diagonal as Ikeno<sup>2</sup> describes for *Marchantia*, and the sperm-cells are not so evidently in pairs; indeed it looks as if there might perhaps be one less division than is the case in *Marchantia*. The spermatozoids are decidedly larger than in any

<sup>1</sup> McFadden, Effie B.: Bull. Torr. Bot. Club, vol. xxiii, 1896, pp. 242-4.

<sup>2</sup> Ikeno, S.: Die Spermatogenese von *Marchantia polymorpha*. Beiheft 3, Bot. Centralbl., vol. xv, 1903, pp. 65-88.

other Marchantiaceae examined by the writer, but there was no indication of any marked difference in their structure from what has been observed in other Hepaticae.

The antheridium as usual is contained in a chamber opening by a narrow pore, and presumably the dehiscence of the antheridium and the discharge of the spermatozoids are effected as in other Marchantiaceae.<sup>1</sup>

### *The Carpocephalum.*

The female receptacle, or carpocephalum, is easily shown to be of the composite type, its pedicel being a prolongation of the axis of the shoot upon which it is borne. Sections through the young receptacle show clearly its compound structure, there being several (6-7) apices, each giving rise to a group of archegonia. The receptacle from the first is convex, and the excessive dorsal growth pushes the growing points below the margin of the receptacle so that the archegonia, which really are dorsal structures, seem to arise from the ventral surface of the receptacle. In short, the growth of the carpocephalum resembles exactly that of the other Marchantiaceae (Text-fig. 5, A, B). The compound nature of the receptacle is sometimes very evident superficially, as its margin may be deeply lobed, each lobe corresponding to a growing point with its group of archegonia. This was especially noticeable in specimens of *D. trichocephala* from the Taiping Hills in the Malay Peninsula (Text-fig. 2, A). The plants were smaller than those collected in Java and the pedicel of the ripe carpocephalum shorter. In *D. velutina* (Text-fig. 3) the pedicel is also decidedly shorter than it is in *D. trichocephala*.

The development of the archegonium is much like that of the other Marchantiaceae. The first division in the mother-cell (Text-fig. 5, C) divides it into a basal tapering stalk-cell and an upper hemispherical cell, which presently undergoes the usual division by three intersecting vertical walls into an axial cell and three peripheral ones. From the former is cut off the primary cap-cell, and the inner cell ultimately produces the egg and the axial row of canal-cells. From the outer cells develop the wall of the venter and the six rows of neck-cells. Sooner or later the cap-cell divides by two intersecting walls, but there are several secondary divisions in the cap-cells, so that more than four cover-cells finally are formed. This is very much like the condition observed in certain species of *Riccia*<sup>2</sup> as well as in a number of other Hepaticae. The cells of the venter-wall show no

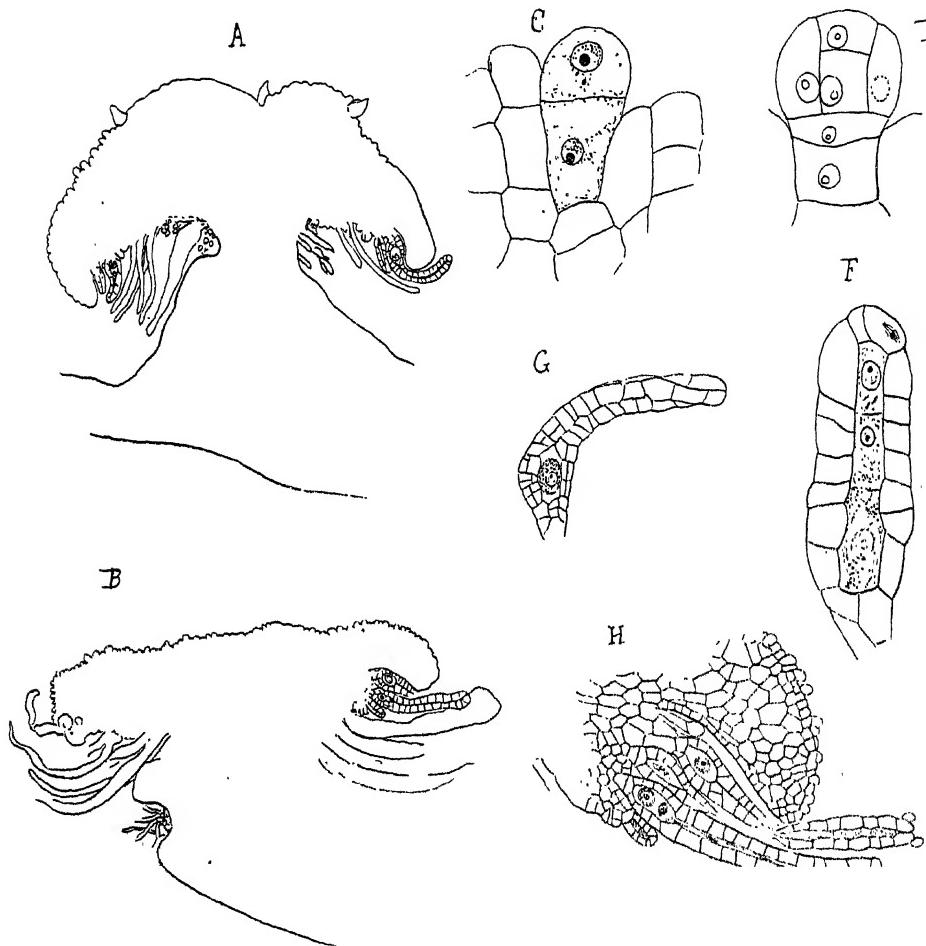
<sup>1</sup> See Peirce, G. J.: Forceful Discharge of Antherozoids in *Asterella Californica*. Bull. Torr. Bot. Club, vol. xxix, 1902, pp. 374-82.

Cavers, F.: Explosive Discharge of Antherozoids in *Fegatella conica*. Ann. of Bot., vol. xvii, 1903, p. 270.

<sup>2</sup> Campbell: loc. cit., p. 30.

periclinal divisions in the mature archegonium. The number of neck canal-cells was not determined with certainty, but is probably six or eight.

The neck of the archegonium in *D. trichocephala* is strongly bent upward when mature, this being probably the result of the position of the



TEXT-FIG. 5. A. Section of young carpocephalum of *D. trichocephala*.  $\times 35$ . B. Similar section of *D. velutina*.  $\times 35$ . C, D. Young archegonia of *D. trichocephala*.  $\times 50$ . E. An older archegonium.  $\times 48$ . F. Ripe archegonium.  $\times 100$ . G, H. Archegonia of *D. velutina*.  $\times 95$ .

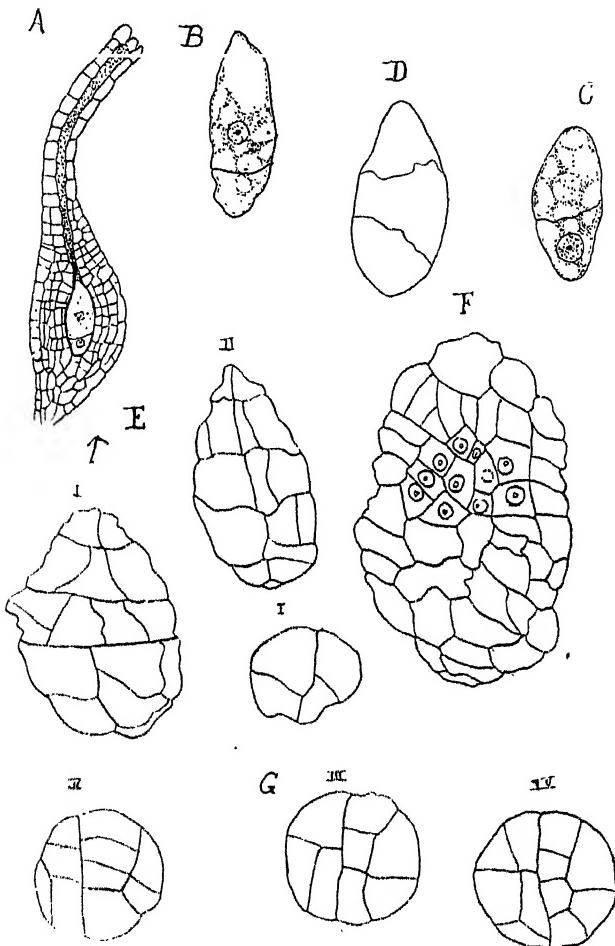
archegonia, which are pushed completely to the lower side of the receptacle by excessive growth of the dorsal tissue (Text-fig. 5, G). In *D. velutina*, where the receptacle is much less convex, the archegonia lie almost horizontally, and the neck is nearly straight (Text-fig. 5, B, H).

No perianth or other envelope is formed about the archegonia. Rhizoids and irregular narrow scales are developed upon the ventral

surface of the receptacle. A cross-section of the pedicel of the receptacle shows two furrows filled with slender rhizoids.

### The Sporophyte.

So far as we have been able to discover, no account of the embryogeny



TEXT-FIG. 6. All figures refer to *D. velutina*. A. Archegonium containing a two-celled embryo.  $\times 115$ . B. Another section of the same embryo.  $\times 285$ . C. A similar embryo.  $\times 285$ . D. Three-celled embryo.  $\times 285$ . E. Two sections of an older embryo.  $\times 285$ . I is a nearly median section. F. An older embryo; the cells of the sporogenous tissue have their nuclei shown.  $\times 285$ . G. Four slightly oblique transverse sections of a young embryo.  $\times 285$ . I is the basal section of the series.

of *Dumortiera* has been published. The recent important work of Meyer,<sup>1</sup> dealing with the embryo of the Marchantiales, does not include *Dumortiera*, nor make any references to the work of others on its embryo.

<sup>1</sup> Meyer, K.: A Study of the Sporophyte of the Marchantiales (Russian). Moscow, 1916.

The earlier stages of the embryo were found only in *D. velutina*; but as the later stages in *D. trichocephala* agree very closely with those in *D. velutina*, it may be assumed that there are no very great differences in the young embryos of the two species.

The fertilized egg, after developing a membrane about itself, becomes elongated and divides by a transverse wall into two cells, of which the upper one is larger than the lower (Text-fig. 6, A, C). The next division is also transverse instead of vertical as is usual in the Marchantiaceae. In this respect *Dumortiera* closely resembles the figures of *Plagiochasma*, described by Meyer.<sup>1</sup> A similar condition has been described for *Targionia* by Miss O'Keefe,<sup>2</sup> but all of the writer's preparations of the young embryos of *Targionia* show the typical quadrant formation.<sup>3</sup>

It is not unlikely that a second transverse wall may be formed in the upper cell of the embryo before any vertical walls appear, but this is not certain. Each of these primary cells is next divided into quadrants by intersecting vertical walls, but these quadrants are not entirely symmetrical (Text-fig. 6, C).

The original hypobasal cell gives rise to the foot, and to part, at least, of the short seta. In this region the divisions are quite irregular (Text-fig. 6, E, F), but in the epibasal portion of the embryo a series of periclinal walls is formed, separating the central sporogenous area from the wall of the future capsule. The terminal segment, however, does not develop any sporogenous tissue, but contributes only to the wall of the capsule, which at the apex is thicker than it is at the sides.

As the young sporophyte grows it becomes oval or pear-shaped in outline, and the sporogenous region or archesporium becomes clearly defined. In this region longitudinal divisions predominate, so that the cells, especially towards the centre of the sporogenous tissue, are much elongated (Text-figs. 7, 8). These cells as usual stain more strongly than the sterile tissues of the sporophyte. The young wall of the capsule consists for the most part of a single layer of cells, but at the apex of the capsule it consists of two or three layers, forming a sort of cap. These parietal cells have less dense contents than the adjacent sporogenous tissue. The latter soon shows a differentiation into spore mother-cells and young elaters. The spore mother-cells are usually in more or less regular rows, due to the repeated transverse divisions of some of the archesporial cells, while the others remain undivided and develop into elaters.

The lower part of the sporophyte develops a short seta merging gradually into a not very well-defined foot. The seta is somewhat better

<sup>1</sup> Loc. cit., Figs. 35, 36.

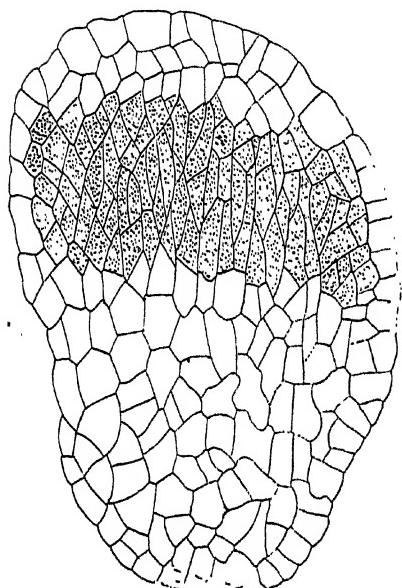
<sup>2</sup> O'Keefe, Lillian : Structure and Development of *Targionia hypophylla*. New Phytologist. vol. xiv, 1915, pp. 105-16.

<sup>3</sup> Campbell : loc. cit., Fig. 23.

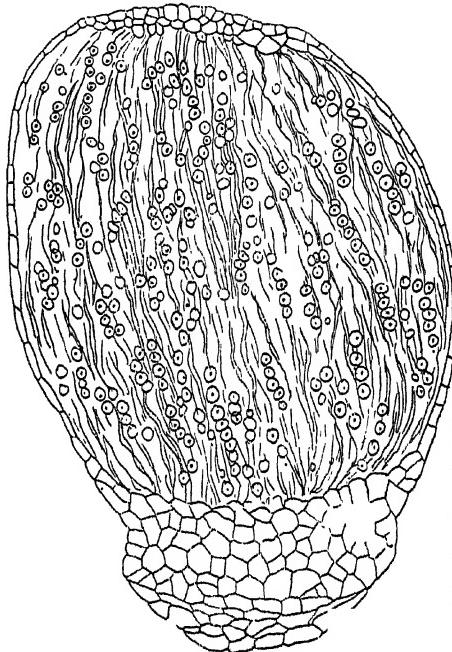
developed in *D. trichoccephala* than it is in *D. velutina*. At maturity there is some elongation of the seta which causes the capsule to protrude completely from the lobe of the receptacle (Text-figs. 1, 2, 3). It opens by four somewhat irregular valves, which are usually more or less split so that the original lobes are not always readily made out (Text-fig. 1, D, E). The parietal cells of the capsule have dark-coloured annular thickenings upon their walls, similar to the spiral bands of the elaters.

The ripe spores of *D. trichoccephala* are somewhat oval in shape, the surface covered with small irregular papillae (Text-fig. 1, F, G). They

A.



B.

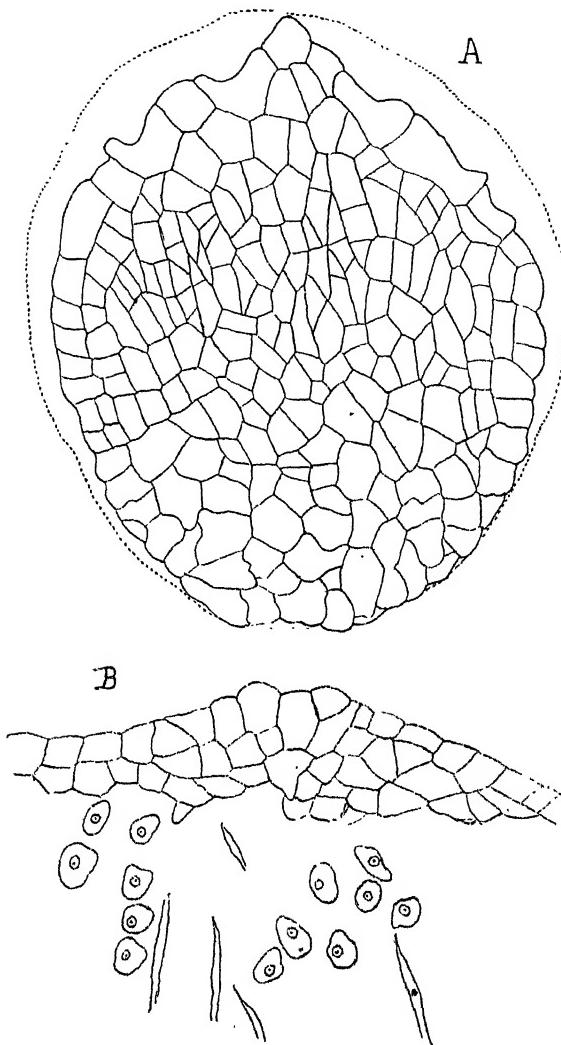


TEXT-FIG. 7. A. Young sporophyte of *D. trichoccephala*.  $\times 255$ . The sporogenous area is shaded. B. An older sporophyte.  $\times 84$ .

measure about  $20 \mu$  in their longer diameter. The spores of *D. velutina* are much like those of *D. trichoccephala*, but are larger, averaging about  $29 \mu$  in length.

As we have already indicated, in its early stages the embryo of *Dumortiera* closely resembles that of *Plagiochasma*. In its later stages, however, both in the form of the sporophyte and the arrangement of the cells in the sporogenous tissue, it is much more like *Preissia* and *Marchantia*.<sup>1</sup> In *Dumortiera* the seta is better developed than in either *Preissia* or *Marchantia*, but the foot is not so clearly defined.

<sup>1</sup> Meyer; loc. cit., Figs. 62, 70, 72.



TEXT-FIG. 8. A. Young sporophyte of *D. velutina*.  $\times 255$ . B. Apex of an older sporophyte, showing spore mother-cells and young elaters.  $\times 255$ .

*Dumortiera calcicola*, sp. nov.

While collecting in Borneo, the writer found what is apparently an undescribed species of *Dumortiera*—certainly very distinct from the two species already considered in this paper. The name *D. calcicola* is proposed for this plant, as it was found in all cases growing upon limestone débris or over outcrops of limestone.

The first specimens collected, which were all sterile, occurred in patches of considerable size upon loose heaps of débris at the foot of the limestone

cliffs in which are excavated the Bidi Caves, not far from Bau, in Sarawak. At Bau other specimens were collected growing on the ground close to outcrops of limestone. These specimens were fertile, that is, they bore both male and female receptacles; but no sporophytes could be found. The substratum where these fertile plants were growing was much moister than the heaps of soft lime-dust upon which the plants first collected were growing, and this probably accounts for their greater luxuriance and the development of receptacles.

The sterile plant (Pl. VIII, Fig. 1, A) is a delicate ribbon-like thallus with a faint midrib. In colour it resembles *D. trichocephala*, a dull olivaceous green, but is very much smaller than that species—or indeed than any other species of *Dumortiera*. The largest sterile specimens measured barely 5 mm. in breadth, while some of the larger forms of *D. trichocephala* may be more than five times as wide (see Text-fig. 1, A).

The fertile plant (Pl. VIII, Fig. 1, B-D, Fig. 2) differs so much in appearance from the sterile one that it was not at first recognized as the same species. The fertile branches are deeply lobed, so that they look as if there was a row of leaves on either side. Corresponding to each pair of lobes is a receptacle, sometimes five or six of these being formed in succession. Both male and female receptacles occur in the series, the first-formed one being usually, at least, male. It is not always possible to determine by a superficial examination whether the receptacle is male or female, but the former is usually more distinctly lobed than the female. So far as could be determined from the material at hand, both male and female receptacles are sessile; but as no sporophytes could be found, although both archegonia and antheridia were abundant, it may be that after fertilization the female receptacle develops a pedicel as in the other species of *Dumortiera*.

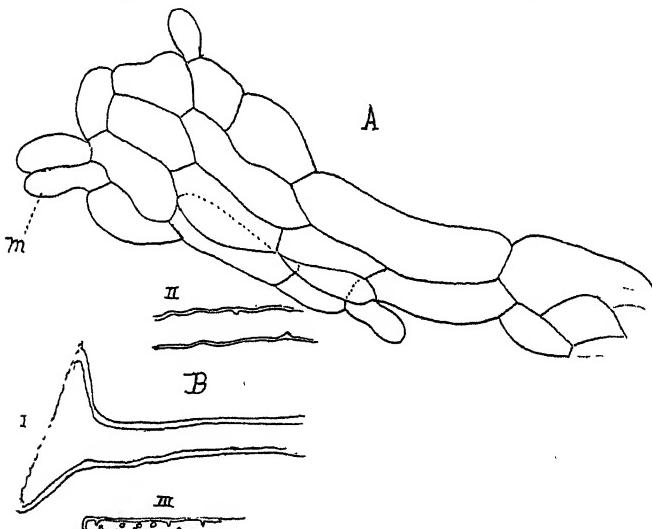
It was supposed when the specimens were collected that the receptacles were really dorsal outgrowths of the thallus; but further examination showed them to be really terminal structures, as in most other Marchantiaceae. Each receptacle terminates a shoot, and all except the first-formed one are borne on short adventitious branches which arise on the ventral side of the next younger shoot, near the apex. These short heart-shaped shoots, each bearing a receptacle at the apex, and closely linked together, give the series the appearance of a single leafy shoot, with a receptacle corresponding to each pair of leaves. Goebel<sup>1</sup> has noted the same phenomenon, though less marked, in the peculiar genus *Monoselenium*; and it is possible that when the sporophyte of *D. calcicola* is found it may show that this plant is more nearly related to *Monoselenium* than it is to *Dumortiera*. It may be said, however, that sometimes in *D. trichocephala* similar adventitious branches are found (Text-fig. 2, B).

<sup>1</sup> Goebel, K.: Archegoniatenstudien, XIII. Flora, vol. cl, 1910, pp. 43-97.

*D. calcicola* sometimes produces androgynous receptacles like those found in *D. trichocephala*.

The branching of the sterile plants of *D. calcicola* is prevailingly dichotomous; but in the fertile plants adventitious branches are sometimes very freely developed in addition to those which bear the receptacles (Pl. VIII, Fig. 2).

A section through the apex of the sterile shoot (Pl. VIII, Fig. 4) shows very clearly the formation of imperfect air-chambers; but, as in other species of *Dumortiera*, these are open above, and no proper stomata are developed. In the older portions of the thallus the air-chambers consist of large shallow depressions whose bounding walls form a faint irregular areolation on the surface of the thallus (Pl. VIII, Fig. 3).



TEXT-FIG. 9. A. Ventral scale. B. Three rhizoids from the thallus of *D. calcicola*.  $\times 285$ .

The short green cell-rows which are so conspicuous on the floor of the air-chambers of *Marchantia* and *Fegatella*, for example, in *D. calcicola* are reduced to rounded papillate cells scattered over the surface of the thallus. These cells are more abundant than in *D. trichocephala*. Like the latter species there are stiff hairs on the margin of the thallus as well as fringing the receptacles, but these hairs are less numerous than in *D. trichocephala*.

A section of the older thallus (Pl. VIII, Fig. 5) shows a pretty well marked superficial layer of small cells, above and below, between which are very much larger cells. In the thinner part of the thallus the latter may be reduced to a single layer. As in all species of *Dumortiera*, the ventral scales are much reduced, and can only be recognized near the apex of the shoot. They are narrow spatulate structures, some of the marginal cells forming pear-shaped glands (Text-fig. 9, A, m).

Rhizoids of three kinds are present—large smooth-walled ones, somewhat smaller ones with occasional tuberculate thickenings, and much smaller ones with numerous tubercles (Text-fig. 9, B).

The most striking character of *D. calcicola* is the arrangement of the receptacles. As has been stated already, male and female receptacles occur upon the same individual, and structurally these do not differ from those of other species of *Dumortiera*. Being of the composite type, the formation of a receptacle necessarily stops the growth of the shoot of which it is a part, and in most species the receptacle is obviously terminal, and only one receptacle is borne on the branch. In *D. calcicola*, however, as we have seen, there are apparently several receptacles formed in succession on the same branch, but this is not really the case. Soon after the first receptacle is formed an adventitious shoot appears close to the apex of the primary shoot on its ventral surface, and this shoot soon develops at its apex another receptacle; and the process may be repeated several times, so that there is a series of short branches, each bearing a receptacle, and so linked together as to give the appearance of a single deeply-lobed thallus bearing a series of dorsal receptacles.

Sometimes two of these adventitious shoots may develop almost simultaneously, thus simulating a dichotomy of the shoot apex (Pl. VIII, Fig. 2). The last-formed adventitious shoot may develop into an elongated sterile branch.

As a rule the primary receptacle bears antheridia, while the later ones are archegonial; but secondary antheridial receptacles are not uncommon, and not infrequently androgynous receptacles occur.

The antheridial receptacle (Pl. VIII, Figs. 6, 7) is usually 5–6 lobed, sometimes quite distinctly triangular in outline (Pl. VIII, Fig. 1, B), while the female receptacle is nearly circular. Both have the margin beset with stiff hairs. On the ventral surface are irregular scales and numerous slender tuberculate rhizoids. The scales are especially noticeable in the younger stages, the subsequent dorsal growth of the receptacle pushing them under so that they are not visible from above.

The male receptacle and antheridia resemble those of the other species, except that they are decidedly smaller and the receptacle is often distinctly lobed. The antheridium has the elongated pedicel observed in the other species.

The young archegonial receptacle is somewhat intermediate in form between that of *D. trichocephala* and *D. velutina*. It is less convex than in the former, but more so than in *D. velutina*. The archegonia have the strongly curved neck observed in *D. trichocephala* (Pl. VIII, Figs. 8, 9).

All of the female receptacles were sessile; but whether this is the case where the archegonia have been fertilized, or whether in such case a pedicel would develop as in all other species, is impossible at present to say, since

for some reason no fertilized archegonia or embryos were present in the material examined.

*Dumortiera calcicola*, sp. nov.

Thallus slender, olivaceous or brownish, 4–5 cm. in length by 4–5 mm. in width. Sterile shoots mostly dichotomous; fertile branches, except the primary ones, arising as adventitious shoots near the apex of the primary branch. Air-chambers evident; dorsal papillate cells rather numerous, but less so than in *D. velutina*. Ventral scales narrowly spatulate, inconspicuous; rhizoids numerous; several (5–6) receptacles formed in succession on a series of terminal adventitious shoots. Antheridial receptacle sessile, often distinctly 5–6 lobed, about 2 mm. in diameter. Archegonial receptacle sessile, similar in form and size to the male receptacle, but less evidently lobed. Sporophyte unknown.

On limestone soil; Bidi Caves, Bau; Sarawak, Borneo, Feb. 1913.

*Wiesnerella denudata* (Mitten), St.

The genus *Wiesnerella* contains a single species, *W. denudata* (Mitten), St.<sup>1</sup> which has been reported from four widely separated regions, viz. Java, the Himalayas, Japan, and Hawaii.<sup>2</sup> Schiffner gave the name *Wiesnerella Javanica* to a Liverwort collected on Mt. Gedeh in Western Java. Stephani considers this to be identical with *Dumortiera denudata*, Mitten, originally described from the Himalayan region. Schiffner<sup>3</sup> has given a clear diagnosis of the plant, but, so far as the writer is aware, did not study in detail the structure of the reproductive organs and sporophyte.

The material upon which the following account is based was collected by the writer near Tjibodas, on Mt. Gedeh, the locality from which Schiffner's specimens came.

*Wiesnerella* (Pl. IX, Fig. 13) in size is about the same as *Dumortiera velutina*, and in general habit is not unlike that species; but the colour is quite different, being a silvery green, quite different from the rich velvety green of *D. velutina*. This difference is due to the presence in *Wiesnerella* of a definite epidermis, with regular pores opening into perfectly developed air-chambers like those in most other Marchantiaceac. These air-chambers at once distinguish *Wiesnerella* from the evidently nearly related genus *Dumortiera*.

A vertical section of the apex of a sterile branch (Pl. IX, Figs. 16, 17) shows much the same structure as that found in *Targionia* or *Fegatella*. The wedge-shaped apical cell appears in this view as a triangle with alternate dorsal and ventral segments cut off from it. As usual, the growth in the dorsal region of the thallus is more active than in the tissue derived from the

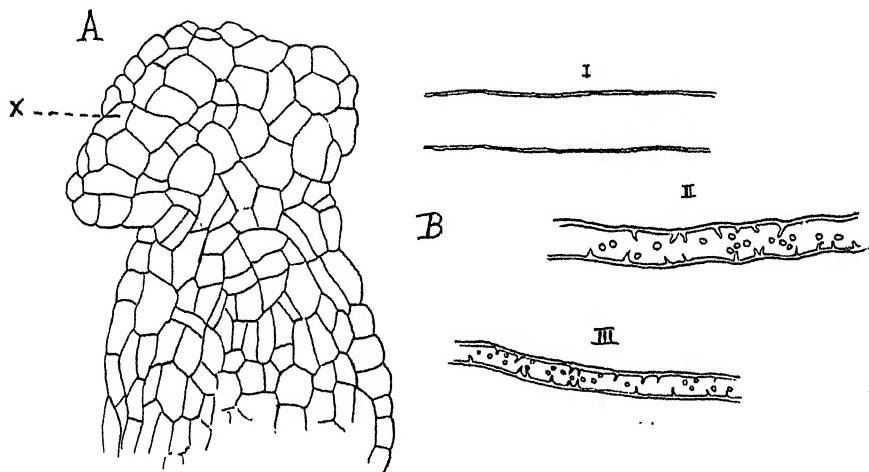
<sup>1</sup> *W. Javanica*, Schiffner.

<sup>2</sup> Stephani: loc. cit., p. 381.

<sup>3</sup> Schiffner: loc. cit., p. 2.

ventral segments of the apical cell. A short distance behind the apical cell, on the dorsal surface of the thallus, small depressions are evident between some of the cells. These are the first evidences of the series of dorsal air-chambers. These in their further development do not show any departure from the type found in *Targionia*<sup>1</sup> and *Fegatella*.<sup>2</sup> The chamber opens at the surface by a simple pore or 'stoma' (Pl. IX, Figs. 19, 20), and the floor of the chamber is occupied by papillate cells, which sometimes show a transverse division. These cells contain a few large chromatophores, and closely resemble those found on the free surface of the thallus in *Dumortiera velutina*.

The ventral scales are better developed than in *Dumortiera*. Each scale consists of a broad basal portion and a nearly semicircular terminal



TEXT-FIG. 10. A. Ventral scale of *Wiesnerella* showing the terminal appendage (x).  $\times 285$ .  
B. Three rhizoids of *Wiesnerella*.  $\times 285$ .

appendage (Text-fig. 10, A). Numerous rhizoids, much like those in *Dumortiera calcicola*, are developed on the ventral surface of the thallus, especially along the axis (Text-fig. 10, B).

*Wiesnerella* is monoecious, the receptacles closely resembling those of *Dumortiera*, but the male receptacles are smaller than in either *D. trichocarpa* or *D. velutina*. The carpocephalum (Pl. IX, Fig. 14) is symmetrically star-shaped, with usually 5–6 rays, and slightly convex. It is borne on a long, slender pedicel.

A few very young receptacles were found, showing that their early development is essentially the same as in *Dumortiera*; but material was not

<sup>1</sup> Campbell : loc. cit., p. 48.

<sup>2</sup> Cavers : On the Structure and Biology of *Fegatella conica*. Ann. of Bot., vol. xviii, 1904, p. 87.

available for a study of the development of the sexual organs, and how far these may differ from those of *Dumortiera* remains to be seen. The adult archegonium, to judge from a small number of unfertilized archegonia that were seen (Pl. IX, Fig. 24), does not differ much from that of *Dumortiera*. The neck is straight, and in this respect it recalls *D. velutina*. As in *Dumortiera*, there is no special envelope or perianth about the archegonia, and the sporophyte, apart from the calyptra, is protected merely by the inrolled margin of the receptacle lobe, which forms a sort of pocket in which the sporophyte is quite concealed until it is ready to open.

The tissues of the carpocephalum are less specialized than is usual among the Marchantiaceae, and in this respect *Wiesnerella* shows its relationship to the still more reduced *Dumortiera*. The dorsal region of the receptacle contains numerous irregular air-chambers, but the stomata are poorly developed (Pl. IX, Fig. 28) when compared with those of most Marchantiaceae, and there are few of them.

In the older receptacles a peculiar phenomenon was noted, which also occurs in a less marked degree in *Dumortiera*. This is a great enlargement of the chromatophores in some of the interior cells (Pl. IX, Fig. 30). Fig. 29 shows a cell with chromatophores of the normal size for comparison. What this hypertrophy of the chromatophores may signify is not clear.

No young embryos were found, and it must for the present remain a question whether they are of the same type found in *Dumortiera*. The older sporophytes (Pl. IX, Figs. 23, 25) are quite similar to those of *Dumortiera*, but the foot is more clearly defined. As in *Dumortiera*, the seta becomes considerably elongated (Pl. IX, Figs. 13, 15), in this respect differing from most Marchantiaceae.

The ripe spores are very different from those of *Dumortiera*. They are very much larger ( $60\ \mu$ ), and instead of the small papillae on the surface there are present very conspicuous wing-like ridges (Pl. IX, Fig. 27).

#### CONCLUSION.

It is pretty generally agreed that the most striking peculiarity of *Dumortiera*, i. e. the more or less complete obliteration of the air-chambers, is secondary and associated with the marked hygrophilous habit of most of the species. Of the species examined, *D. velutina* shows the least reduction, for not only are the outlines of the air-chambers quite evident, but the characteristic assimilative tissue is present in the form of very numerous superficial papillate cells. In *D. trichocephala*, which is more strongly hygrophilous in its habit, the reduction of the air-chambers is much more complete, and in one Hawaiian species (assumed to be *D. trichocephala*) the suppression is complete, as it is in the presumably related genus *Moneses*.

*Wiesnerella* is especially interesting, as it is on the one hand unmistakably closely related to *Dumontiera*, while on the other it is obviously connected with the typical Marchantiaceae. It may be fairly said to connect forms of the type of *Marchantia* with the reduced *Dumontiera*. About the only evidence of reduction in *Wiesnerella* is the character of the stomata, especially those upon the receptacle.

The writer is indebted to Professor H. L. Lyon of Honolulu for mature specimens of the two Hawaiian species of *Dumontiera*. The carpocephala and spores of the smooth form are indistinguishable from those of the East Indian *D. trichoccephala*. The other species is evidently not *D. velutina*, as suggested by the writer, but is probably *D. hirsuta*, which Stephani records for Hawaii.

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## DESCRIPTION OF PLATES VIII AND IX.

Illustrating Professor Campbell's paper on Studies on some East Indian Hepaticae.

### PLATE VIII.

All figures refer to *Dumontiera calcicola*, sp. nov.

- Fig. 1. A, sterile; B-D, fertile plants.  $\times 2$ .  
Fig. 2. Fertile plant, showing extensive adventive branching.  $\times 2$ .  
Fig. 3. Apex of a sterile shoot, showing the outlines of the air-chambers.  $\times 25$ .  
Fig. 4. Vertical section of the shoot-apex.  $\times 380$ . x, apical cell; L-L, air-chambers; sc., ventral scale.  
Fig. 5. Vertical section of the thallus.  $\times 380$ .  
Fig. 6. Longitudinal section of a shoot, showing antheridial and young archegonial receptacles.  $\times 50$ .  
Fig. 7. Outline of antheridial receptacle.  $\times 25$ .  
Fig. 8. Section of young archegonial receptacle.  $\times 92$ .  
Figs. 9-10. Archegonia.  $\times 380$ .  
Fig. 11. Outline of young archegonial receptacle.  $\times 25$ . sc., ventral scales.  
Fig. 12. Antheridia.  $\times 95$ .

### PLATE IX.

All figures refer to *Wiesnerella*.

- Fig. 13. Fertile specimen.  $\times 2$ .  
Fig. 14. Carpocephalum, seen from above.  $\times 3$ .  
Fig. 15. Open sporogonium.  $\times 5$ .  
Fig. 16. Median longitudinal section of the thallus, passing through the apex.\*  $\times 125$ .  
Fig. 17. Apex of a shoot, showing the formation of the air-chambers, L.  $\times 640$ . x, apical cell.  
Fig. 18. Horizontal section of thallus-apex.  
Fig. 19. Vertical section of air-chamber and stoma.  $\times 380$ .

Fig. 20. Surface view of a stoma.

Fig. 21. Section of young carpocephalum.  $\times 380$ . *ar.*, young archegonium.

Fig. 22. Section of an older carpocephalum.  $\times 58$ .

Fig. 23. Nearly ripe carpocephalum, showing a sporophyte, *sph.*, enclosed in the calyptrae. *ar.*, unfertilized archegonia.  $\times 50$ .

Fig. 24. An unfertilized archegonium.  $\times 140$ .

Fig. 25. Nearly mature sporophyte.  $\times 58$ . *d*, thickened apical portion of the wall.

Fig. 26. Apical region of a nearly ripe sporogonium.  $\times 95$ .

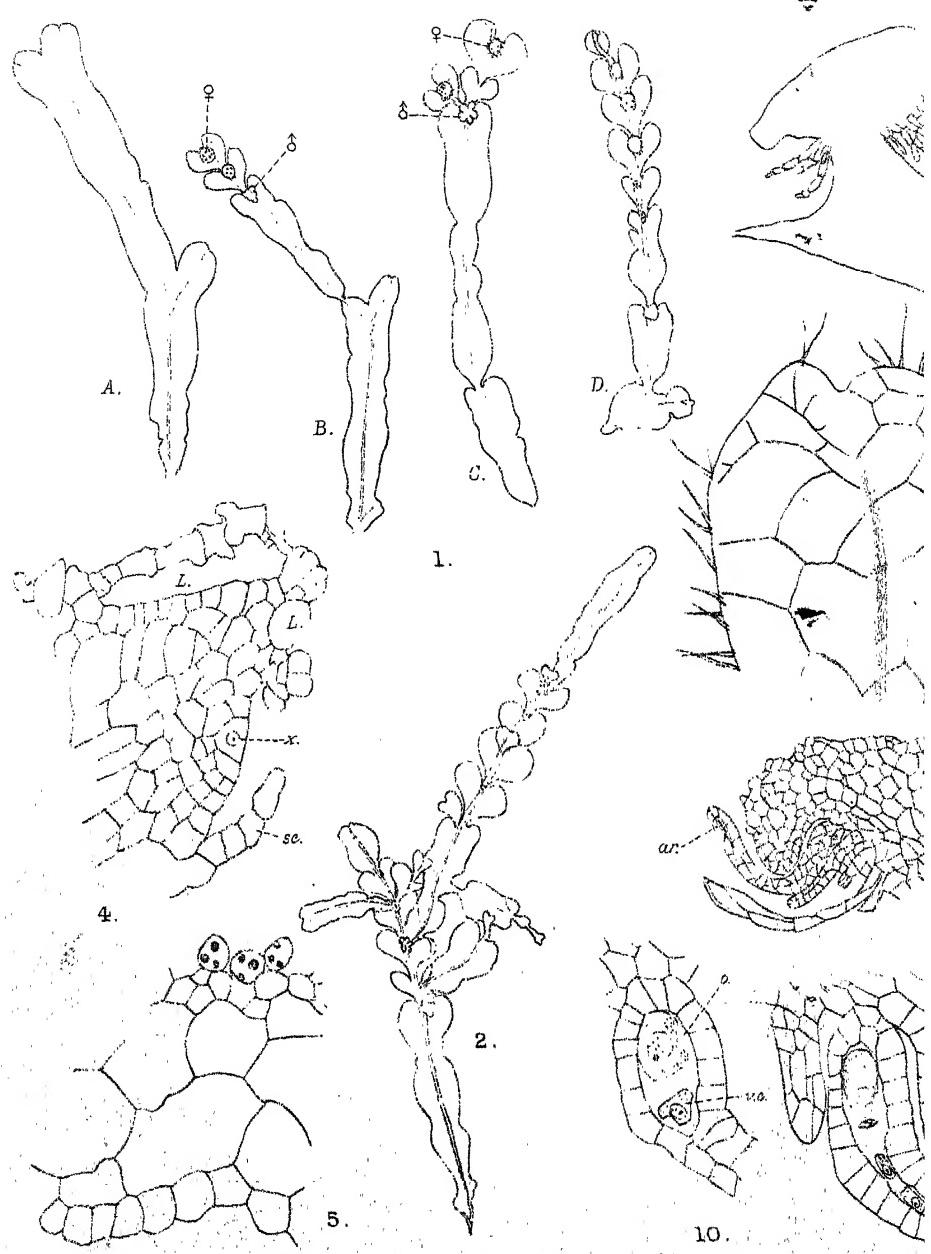
Fig. 27. A ripe spore and part of an elater.  $\times 640$ .

Fig. 28. Stoma from the carpocephalum.  $\times 95$ .

Fig. 29. A cell from the carpocephalum, showing normal chromatophores.  $\times 690$ .

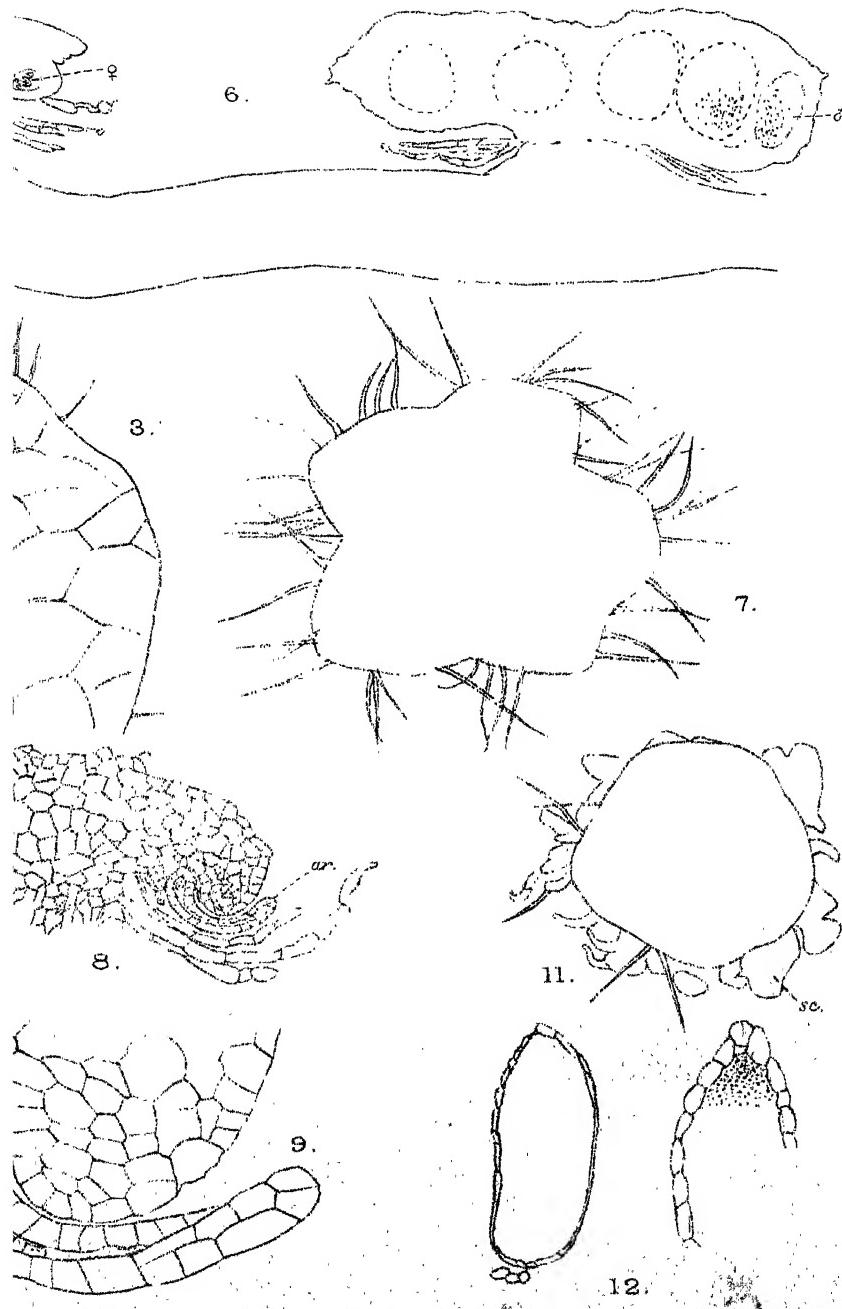
Fig. 30. Cell from the carpocephalum, showing much enlarged chromatophores.  $\times 640$ .

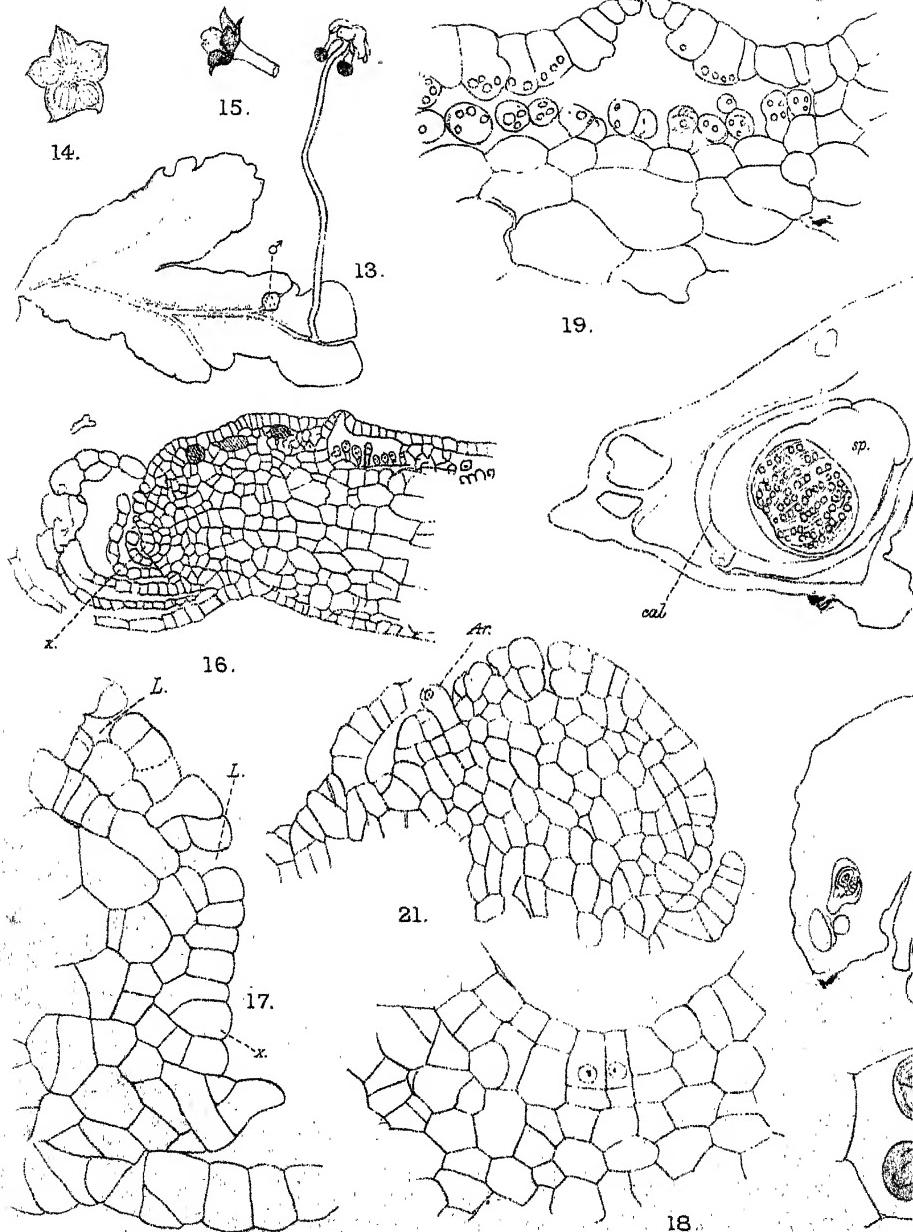




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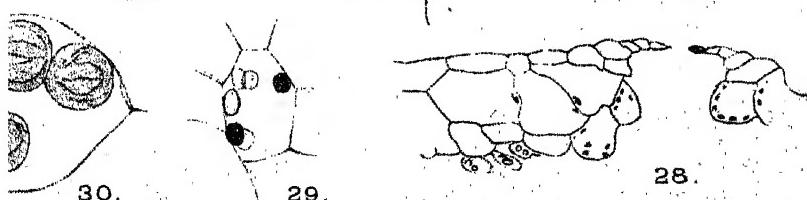
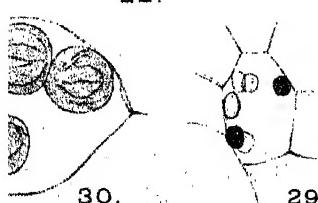
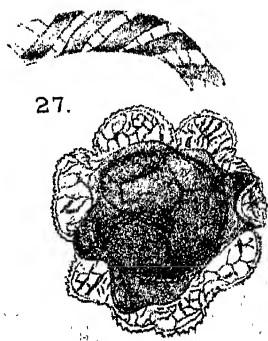
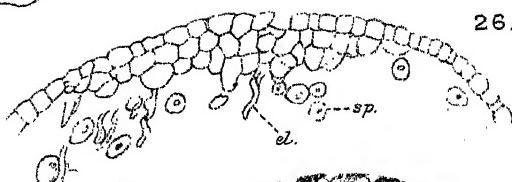
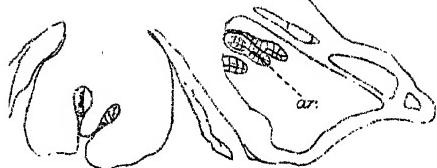
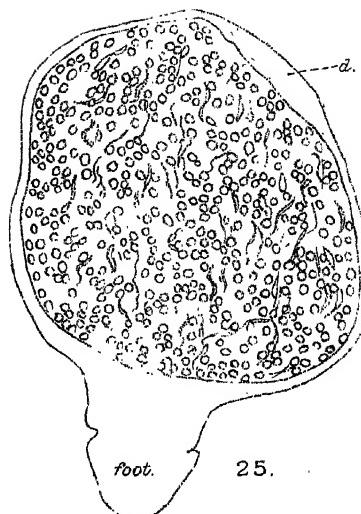
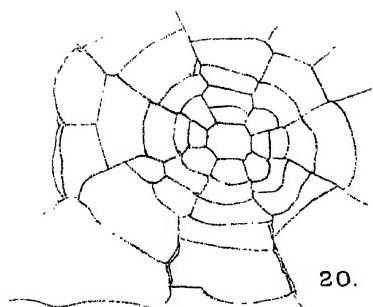
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# The Sources and Distribution of the New Zealand Flora, with a Reply to Criticism.

BY

J. C. WILLIS, M.A., Sc.D.

With seven Figures and Map in the Text.

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A NUMBER of reviews and criticisms have lately appeared, supporting or attacking my hypothesis of 'age and area'. Two (11, 12) by Professor Sinnott are of particular interest, and follow lines which he has more fully developed in earlier publications (13, 14). In replying to these criticisms I shall endeavour, as much as possible, to use new facts, or new presentments of facts, about the flora of New Zealand and its surrounding islands, a flora which is of great interest when examined in the light of age and area.

The employment of this hypothesis suggests new methods of attacking the facts of geographical distribution, and has led to the discovery of clear evidence in favour of certain hypotheses which in the past have been the subject of much discussion. It is, for example, as will be seen below, rendered probable that the peopling of New Zealand with plants was by way of land bridges, and that there were at least two of these, one northern, leading to some part of Indo-Malaya, and one southern. By the former the arrivals were mainly trees and shrubs, by the latter herbs. I regret that my reply must to some extent take the form of a criticism of the very interesting hypotheses that Professor Sinnott has brought forward in his various papers, but they are too important in their general bearings upon botanical and evolutionary science to pass without comment, especially as they do not rest upon definite proof, but upon a masterly presentment of probabilities.

Professor Sinnott's chief points, as I understand his papers, are :

(1) That my hypothesis is an assumption.

[*Annals of Botany, Vol. XXXII. No. CXXVII. July, 1918.*]

(2) That I have not allowed enough for other factors that determine distribution.

(3) That the bulk of endemic species are 'relicts' and not new appearances, i. e. that they are in general older than the wides that are mingled with them; 'very many endemics owe their limited distribution to the circumstance that they are remnants of comparatively unsuccessful types which have been exterminated elsewhere, and which even in these isolated floras are waging a losing fight against more vigorous and adaptable new-comers.'

(4) That trees and shrubs are in general older than herbs.

(5) That age tends to the disappearance of old species. He 'regards isolation as a factor which tends not only to develop new species, but also to modify and extinguish old ones; and hence looks upon species in Ceylon and New Zealand which still maintain specific identity with their co-types on the mainland as the newest arrivals rather than as the most ancient members of the flora'.

(6) That age and area fails to explain the distribution of the New Zealand flora.

(7) Certain minor criticisms near bottom of p. 214.

I must begin by pointing out one or two instances in his papers where Dr. Sinnott shows that he has not quite clearly grasped the exact meaning of my work—no doubt on account of my imperfect presentation of it. In the first paragraph of his paper (11) he quotes my hypothesis without the very important proviso that it be only applied in cases of about twenty allied species, and then goes on to argue as if it were intended to apply in individual cases. He states that 'a highly specialized form, occupying a relatively narrow ecological niche, may in reality be much older than one which from its greater adaptability under diverse environments is able to thrive over a wider area'. Perfectly true, but it is in the highest degree unlikely that he would find twenty allied forms, or even a whole genus of more than five or six species, living in the same ecological niche, and in the rare cases where this does occur, one would not reason as to age.

On p. 215 he quotes the facts upon which I have founded my hypothesis, but omits the very important detail that not only is the area occupied by the wides greater, but they show their maximum number occupying the largest area, and the numbers are graduated down from this. The endemics, on the other hand, usually show the maximum number on the smallest area, and the numbers are graduated in the reverse direction, unless, as in the case of the species endemic to New Zealand and the islands, or the fern endemics, they are also very old. He likewise omits the important detail, which was what led me first to form the hypothesis, that the species of Ceylon and Peninsular India (and the same is true of those of New Zealand and islands) were on the whole intermediate in area occupied between those

of Ceylon only and those of wider distribution than Ceylon and southern India.

Professor Sinnott seems to think that I am propounding age and area as a kind of master-key that is to unlock everything. Naturally I have laid most stress upon it in my papers, for I am trying to get it established as a law. But it would be as reasonable to try to explain everything by it as to try to explain the upward movement of an aeroplane or a balloon by appeal to the law of gravity. What I am endeavouring to make clear is that, though plants (and I am inclined to think that it applies to animals also) are determined in their existing ultimate distribution by the operation of very numerous causes, they all obey as much as possible the law of age and area, which shows quite clearly in the figures of distribution of any group of plants, even though it may not always show in individual cases.

Contentions based upon probabilities cannot weigh so heavily against age and area as the very clear and decisive figures which have appeared weigh in its favour. These figures, it must not be forgotten, do not depend in any way whatsoever upon the acceptance or rejection of the hypothesis, but simply represent the plain unvarnished facts of distribution. They were discovered by aid of age and area, it is true (except the first ones relating to Ceylon, which originally suggested age and area), but that was simply because the hypothesis acted as a guide to directions in which to seek. This alone is a very powerful argument in its favour, that by its use one is able to discover new facts and new methods of looking at them, which may lead to advances in our knowledge of geographical distribution. It is quite impossible to predict by aid of Natural Selection what will be the actual facts of the geographical distribution of any plants in any country. For instance, one cannot predict the distribution in New Zealand of the plants of the Chathams, or that, to quote de Vries, 'the endemics with a small distribution are heaped up in the centre of the country', or again, that an endemic in New Zealand will occupy a greater area than in Ceylon.

Dr. Sinnott is inclined to say that 'so-and-so must be so', but these statements are sometimes rested upon assumptions which are rather difficult to prove. On p. 210, for example, he says, 'a species with means for rapid dispersal will evidently overrun a wider area in a given length of time than will a more slowly moving type', and on p. 214, speaking of new arrivals, he says, 'after its first rapid spread', therein assuming that under untouched natural conditions that spread is rapid. The facts at our disposal do not warrant such assumptions. We have all but no information as to spread under untouched natural conditions; the only material available refers to spread under conditions altered by man, whose interference in a country may rapidly become of supreme importance to dispersal of plants or animals therein.

In my 'Catalogue of Ceylon Plants' (15) is a list of 387 species known to have been introduced and more or less naturalized there, and the rapid spread of some of which, e.g. *Lantana*, has been used by the supporters of Natural Selection as an argument for the greater adaptability of foreign species to local conditions. In a recent paper (20, p. 197), I pointed out that this assumption ignores three important facts, (1) that foreign conditions have also been introduced, (2) that such weeds are also common in continental areas, and (3) that they spread just as much at the expense of the wades already in the country as of the endemics. We may supplement this by a brief analysis of the list. Of the 387, 204 are cultivated only, 47 are semi-wild cultivated plants found only near houses and gardens, and 125 are weeds of open ground, a feature which was almost unknown in Ceylon before the advent of man. This leaves only 11, of which two have only been once recorded, and have not been seen in recent times, and five exist only as clumps of two or three planted trees. There are thus only four left, of which *Passiflora edulis* might almost be added to the semi-wilds, as it is only found fairly near to places where it is or has been cultivated. *Bocconia cordata* and *Sapum sebiferum* have spread a few hundred yards down-stream from Hakgala Botanic Garden, and there remains only *Aloe vera* var. *littoralis*, which is common on the northern coast, and which is quite possibly a real native of Ceylon. The introduced plants of Ceylon are thus plants which are better adapted to the new conditions created by man, but that is all.

Even in the cases recorded of rapid spread of introduced plants, those without special mechanisms have often been dispersed just as rapidly as those with such. None of the Ceylon introductions has spread more quickly than *Tithonia diversifolia* (Compositae), which has no pappus, and has spread largely by vegetative methods. *Elodea* in western Europe was a similar case, and there are several others. It would seem *a priori* probable that the possession of means for dispersal should improve the chances of rapid spread, but there are few facts to support this view. An examination of the 'adaptations' for dispersal shows at once that they are usually confined to very small groups, and are therefore not very old, regarded from the general evolutionary point of view. In Compositae, practically the whole family has the same mechanism, and as it is usually regarded as young, it may be that its wide distribution in the comparatively short time is due to its possession of such a mechanism.

Dr. Sinnott, in bringing up objections to my hypothesis, to which he gives a much wider application than I have yet claimed for it, passes over without mention such extraordinary results as those given in the Tables IV, V, and VI of my New Zealand paper (19). It is perfectly obvious that such results must be explained; and it is almost equally obvious that no explanation other than that they are the result of a mechanical cause will

meet the necessities of the case. If the endemics are dying out, then they are dying out in a purely mechanical way, in 'wheels within wheels', whether they have or have not wides of the same genus beside them.

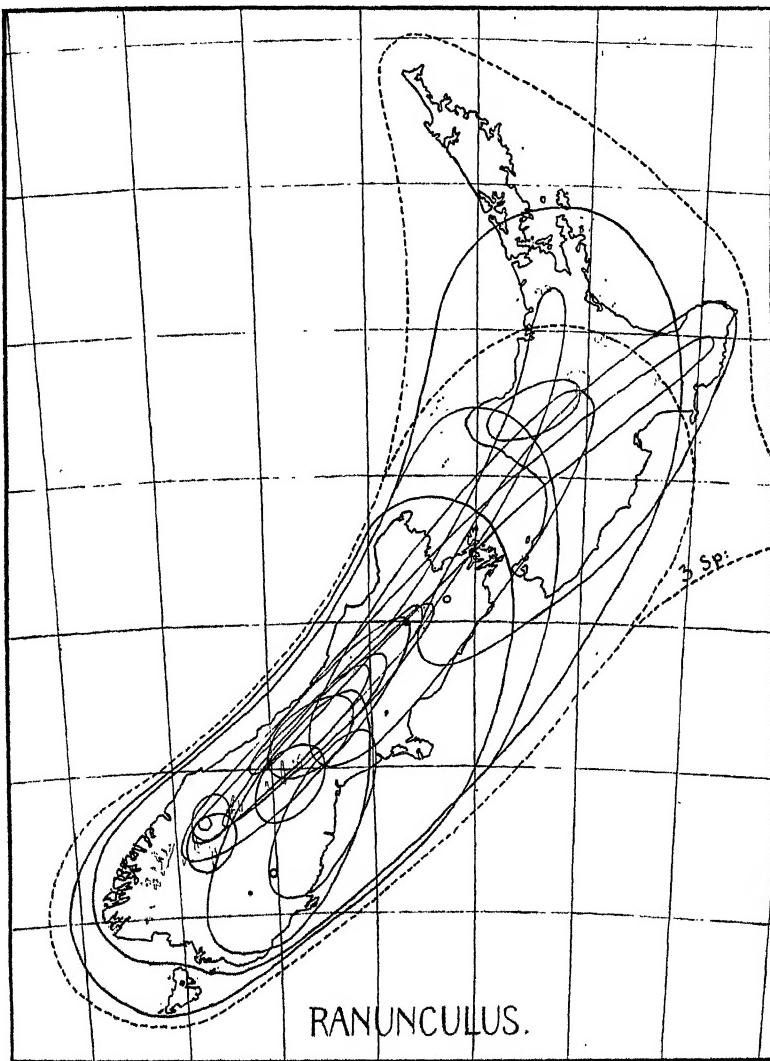


FIG. I.

Wides dotted; extension N. includes Kermadecs, E. Chathams.

Every single family and genus in the New Zealand flora shows one of two types of arithmetical arrangement in these tables. Either, as in Ranunculaceae, the endemic species occurring in each successive zone of 100 miles vary from a small figure up to a maximum and then down again,

or, like Pittosporaceae, they begin with their maximum to the north, and decrease southwards.

To save the trouble of reference I quote the figures for these two families :

	1-100 m.	101-200 m.	201-300 m.	301-400 m.	401-500 m.	501-600 m.	601-700 m.	701-800 m.	801-900 m.	901-1000 m.	1001-1600 m.
<i>Ranunc.</i>	3	7	8	13	16	21	21	28	26	15	3
<i>Pittosp.</i>	11	11	11	11	8	7	6	6	5	5	1

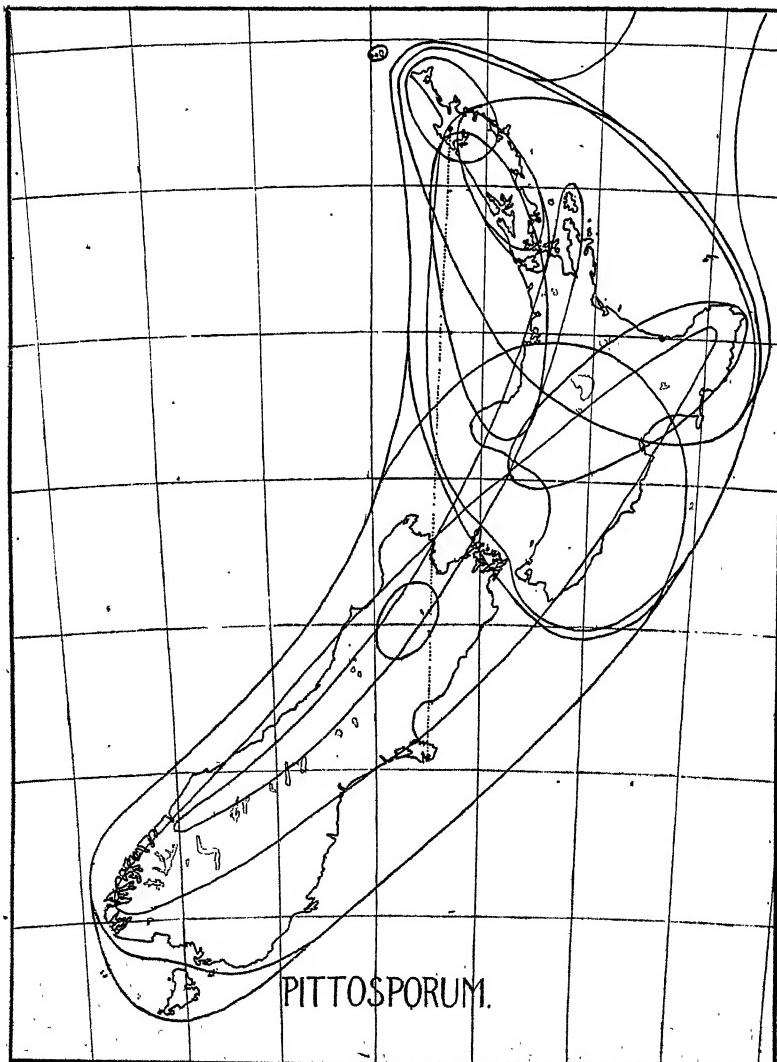


FIG. 2.

As I find that many biologists are not familiar with the method of handling these problems by aid of statistics, so that rows of such figures do not bring the facts clearly before them, I have in the diagrams 1-5 presented the result by a graphic method, showing roughly, by 'circles' drawn round their outermost localities, the actual longitudinal range of the

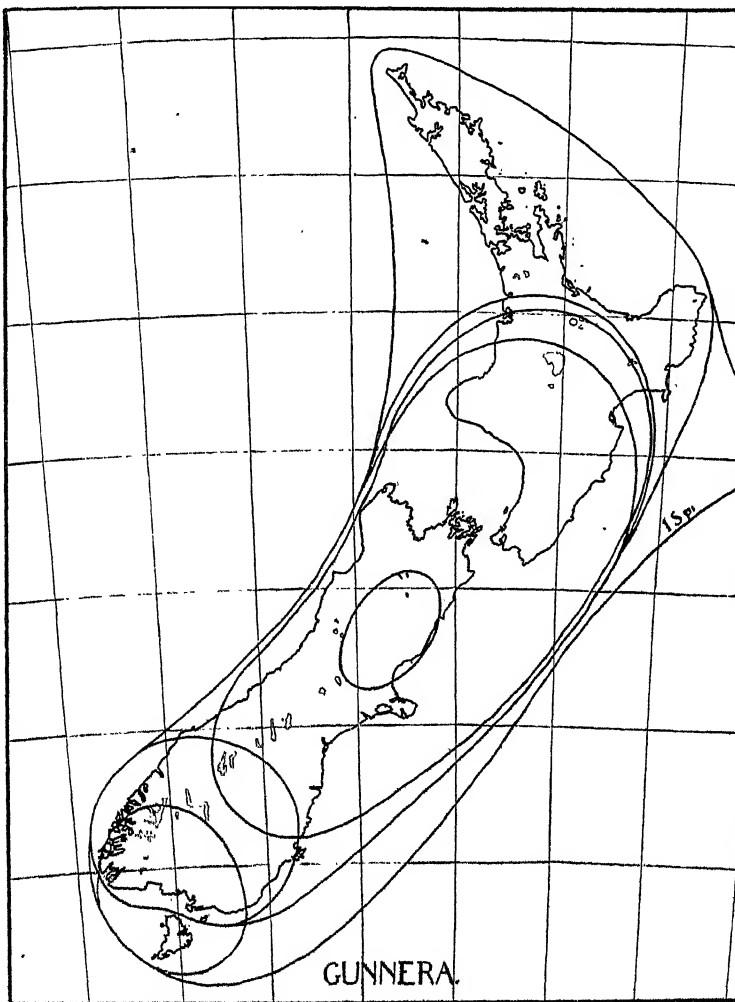


FIG. 3.

species in *Ranunculus* (genus with both wides and endemics, and southern maximum; herbs), *Pittosporum* (endemics only, northern maximum; trees and shrubs), *Gunnera* (endemics only, southern maximum; herbs), and *Cotula* (wides and endemics, southern maximum; herbs), as well as *Haastia* (endemic genus, southern maximum; herbs). But it must be

remembered that every single genus in the whole flora would give such a diagram, reminding one of the ripples made by throwing a stone into water, and it seems to me difficult to maintain, in face of such facts, that the distribution of the species is not chiefly determined by a mechanical cause.

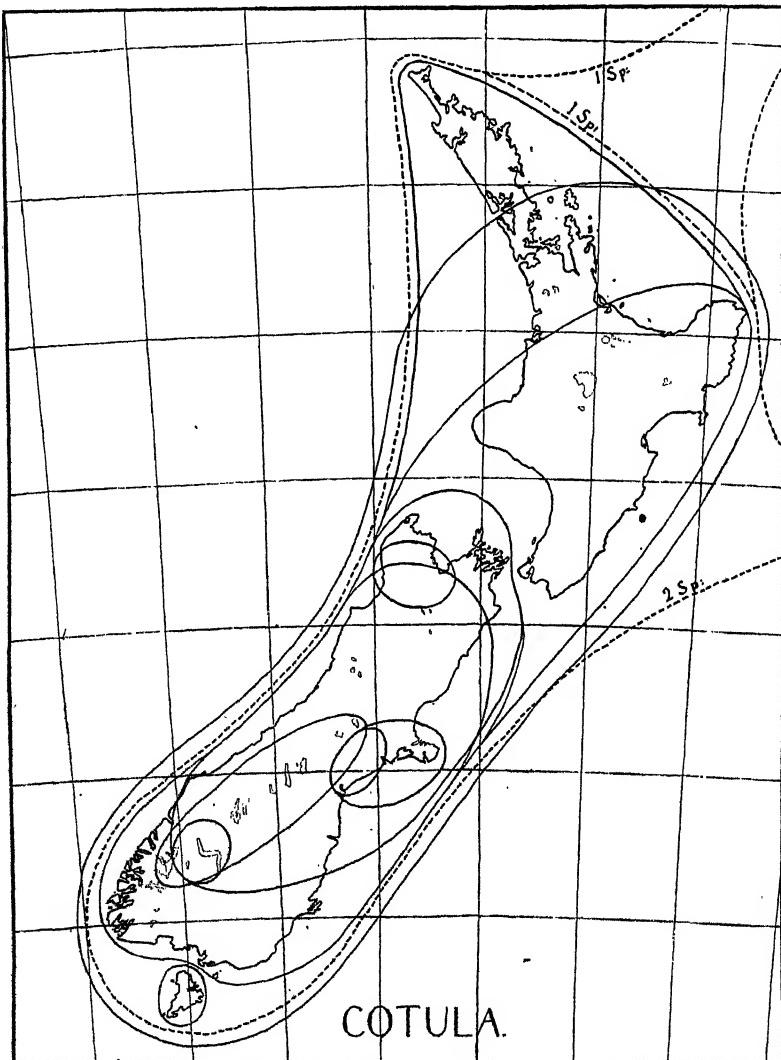


FIG. 4.

This cause I believe to be simply age. Whether the genus be Monocotyledon or Dicotyledon, tree, shrub, or herb, endemic or not, composed of wides and endemic species, or of endemics only, of Indo-Malayan, Australian, or South American affinity, it behaves in the same way.

To turn now to the principal points in Dr. Sinnott's criticisms, as given above, the first is that age and area is an assumption. Perfectly true, but so is Natural Selection, and it is no more improbable that area occupied should increase with age than that a well-equipped plant *A* should beat a less well-equipped *B* in the struggle for existence. Both seem fairly self-

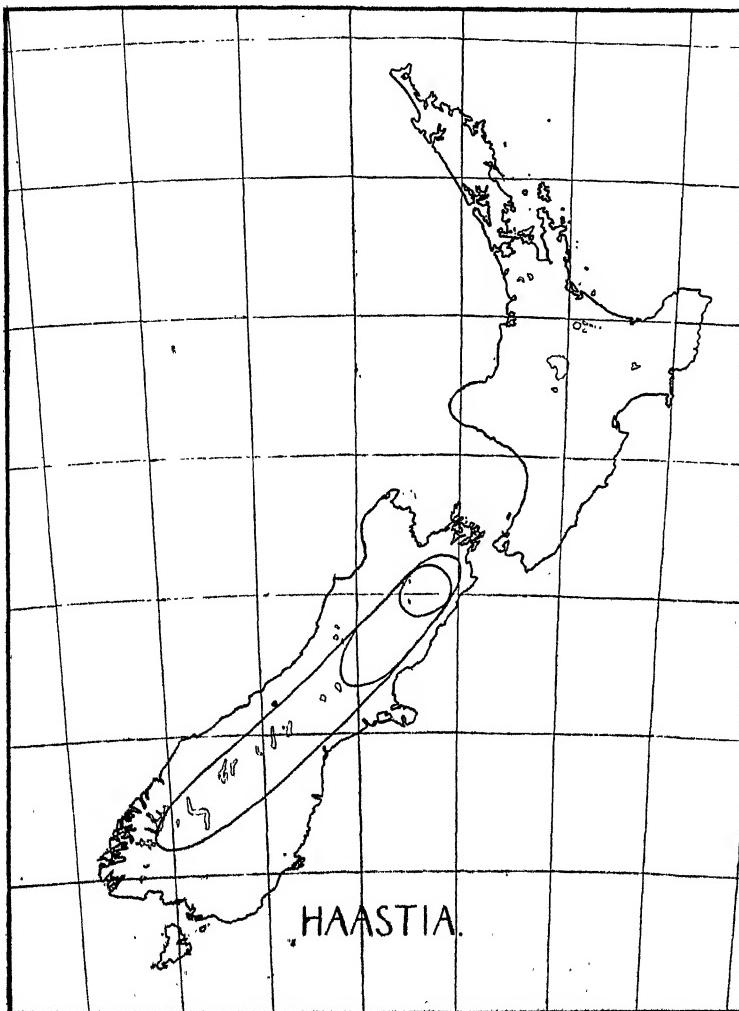


FIG. 5.

evident truths. But for the last fifty years Natural Selection, to the practical exclusion of everything else, has been regarded as the chief operative factor in evolution and geographical distribution. We are still, however, without any proof that it determines the area occupied by species, whereas actual arithmetical results of the clearest kind, which are rapidly accumulating,

speak in favour of age and area. Further, as I have pointed out above, prediction is possible under age and area, and impossible under Natural Selection, and, so far, all predictions made have been verified by the actual facts.

The objection to 'age and area' that many people profess, when analysed, is really an objection to changing the mode of looking at certain facts. But, as I have already pointed out, the new method is *a priori* just as likely to be correct as the old, and in any case it is a good thing sometimes to change one's point of view, even if it be only for a time.

#### OTHER FACTORS THAN AGE ACTIVE.

Dr. Sinnott objects that I have not allowed enough for the action of other factors than age. I have never pretended to exclude them from operation; in my paper on Ceylon (18), p. 5, I wrote, 'of course in the case of any single species numerous disturbing influences come into play', and in the New Zealand paper I called attention to the effects of man's action, change of climate, &c. In my reply to Mr. Ridley (20), which Dr. Sinnott had not seen when he wrote, I have gone more fully into the question of other determinative factors in distribution, and have given a list of such factors, which is being steadily added to.

The figures I have given, however, show that age and area is a law which appears to affect all plants taken in groups of allied species more or less closely alike, and this cannot be said of any of the other causes. Probably in every single case of an individual species one or more of them come into action; they do not, however, act alike on groups of species, but pull every way, so that when groups of allied species are considered, as should always be the case in using 'age and area', their results cannot be clearly seen.

Hydrogen rises in air; an aeroplane rises; a piece of paper falls slowly; a rifle bullet falls at an angle with the soil; yet the law of gravity is recognized as universally applicable in all these cases, though other factors may be acting so strongly as to conceal its operation. No one doubts the validity of Mendel's law because in almost no single case does the progeny appear in the exact proportions required by the law; and similarly with age and area, I believe it to be a general law, though its action may be concealed in individual cases by the operations of one or more of the other factors which have some effect in determining geographical distribution.

Age and area shows just as clearly in the Ferns as in the Angiosperms, and in both shows family by family and genus by genus, so that it is obviously a very ancient law, whereas the other factors are mostly such as only come into operation in individual cases, or, as in the case of Natural Selection, exert a differentiating action, with results which do not show in the figures for geographical distribution.

## ARE ENDEMICS CHIEFLY RELICTS?

Dr. Sinnott takes the popular view, which is based, it must be remembered, upon an assumed efficacy of Natural Selection for which as yet there is little proof, that species with small areas of distribution owe the fact that those areas are small to the competition of other more successful types. But there is little evidence for such a belief. It is simply a way of looking at the actual fact, which is all we have to go upon, that *A* occupies a large and *B* a small area. My way of looking at the same fact is to suppose that *A* is older than *B*. This is really a much more simple explanation, especially when we remember that the areas occupied by the different species in a genus, or the different genera in a family, usually increase fairly regularly from very small to large. If one have areas represented by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, it seems an unnecessarily oblique way of looking at the facts to say that 1, 2, 3, 4, and 5 must be regarded as dying out, while 16 to 20 are to be looked upon as successful and expanding species, and no two authors can agree about whether the intermediate species 6 to 15 are one thing or the other. It is far more simple to regard all as still in process of expansion, but that some, by reason of greater age and perhaps other advantages, have grown larger than others.

Not only is this explanation simpler, but predictions can be based upon it, a thing which was impossible with Natural Selection. I have already based quite a number of predictions upon my hypothesis of age and area, and have shown that they are verified when the actual facts come to be examined. Now it seems to me that when one has to consider the acceptance of an hypothesis which admits of prediction, and when the predictions made with its aid lead at once to the discovery of new facts hitherto unknown, and confirming the hypothesis, the balance of probability is likely to be in its favour.

Dr. Sinnott says, on p. 212 (11), that I disregard the evidence that many endemics are not of local origin, but are relicts. Here it seems to me that from giving special attention to cases of extinction he is apt to forget that the number of examples in which this has been shown to be likely is comparatively small. I very much doubt if it can be regarded as probable for even 1 per cent. of the endemic species of the world. There are certainly not ten cases in New Zealand, and the islands contain over 900 endemic flowering plants. Age and area simply shows that practically all endemic species in a given country behave in the same way, but cannot easily distinguish between those which were actually formed *de novo* in the country, and those that may have come in from outside over land now submerged. But if these species are endemic, then they must have originated, if not within

the country, at least not very far away. And often the local distribution enables one to decide.

The same criticism applies to the remark lower down on the same page, that 'we are familiar with many species, the range of which is widely discontinuous'. Here, again, the total number, though in itself considerable, is really very small when compared to those with continuous ranges. Again he says, dealing with the species of Ceylon (wides) which have discontinuous ranges and have co-types in Assam, and New Zealand wides which have co-types in South America, that a little more dying out would result in the production of forms definitely endemic in one of their present areas. Quite true, but he has to show that such a thing as *dying out* without change of conditions can occur. On my view a species may be *killed* out by submergence, great climatic change, or other catastrophe, but will continue to spread (following age and area) in the regions where it survives, so long as conditions there remain unaltered. In the case of the New Zealand species, the antarctic land that once connected them to South America has either disappeared or become incapable of supporting plant-life, and in the case of Ceylon and Assam there is good reason to suppose that the two were for some time separated by an arm of the sea, while in recent times, at any rate, the intermediate country has been dry, Ceylon and Assam being both wet. Though cut off from their co-types, the species of Ceylon and New Zealand behave in those countries exactly like the other species found there, and are arranged in graduated series in 'wheels within wheels'. It is impossible to reconcile the idea that *many* endemics are dying out with the regular graduation of species shown by my figures. One cannot conceive of species dying out in such a regular way, whether they have or have not wides beside them, whether they are endemic species in wide genera, or endemic genera, whether endemic with large or with small area, and the rest (cf. the distribution map of Doona (18, p. 14) and those given above).

Can Dr. Sinnott produce, on the hypothesis of Natural Selection, any shadow of a reason why *Ranunculus Lyallii*, perhaps the finest of the Ranunculi, should be confined to South and Stewart Islands in New Zealand, while *R. acaulis* and *R. rivularis* (wides) range New Zealand from end to end and reach the Chathams, and several endemics range into the North Island also? Other reason, that is, than the mere fact that such is the case. The natural selectionists assume, without facts to go upon, that this and other endemic species are dying out by reason of unsuitability. But in no single case can they say with certainty that a species is really dying out under unchanged conditions. In this particular case, for example, some would probably maintain that it was dying out, others would vehemently deny it. They cannot in the least define a size of area above which species are to be regarded as growing, or below which as dying out, and would apparently prefer to see the subject of the distributional areas

remain as incomprehensible, just as the special creationists considered was the case with the fact that such and such species occurred in one country, such and such in another.

The position of the natural selectionists is based upon assumption, whereas age and area is already supported by numerous incontrovertible facts, which are being rapidly increased by new work, and every prediction as yet made by its aid has been verified. Why does not *Ranunculus Lyallii* occur in the North Island, where there are fewer Ranunculi to compete with, fewer species of every kind, a less strenuous competition generally. Natural Selection cannot hope to explain this fact, but can only accept it as a fact, whereas age and area simply explains it as being so because it was not evolved in time to reach the North Island before the formation of Cook's Strait.

In many cases, no doubt, but in few compared to those in which it is not so, there is a certain amount of geological evidence of former greater spread, but that is no evidence that the species is dying out where it now exists, unless man has altered the conditions. It is almost impossible at the present juncture to lay too much stress upon the influence of man, for Natural Selection has been so largely supported upon evidence of what has happened under that influence, without any evidence that it would happen under unchanged conditions.

I have already been very fully into this question, but as it is the principal argument brought forward by those who are opposed to age and area, it may be as well to bring up other points in this connexion. I may commence by enumerating the chief points of a recent paper (20).

(1) The regular arrangement of my figures demands that youth be substituted in detail for age in my hypothesis, if endemics are to be regarded as older. This may be youth within the country, or absolute youth.

(2) Absolute youth is somewhat discredited by the fact that range within the country does not depend upon range outside.

(3) Youth within the country—the logical reversal of age and area—has no conceivable connexion with area occupied, and leads to various absurdities, besides involving much rising and falling in the scale of commonness (area of distribution) for which we have no warrant.

(4) The dying out (assuming that it is occurring) of the endemics is purely mechanical, every family and genus behaving in the same way, whether it has or has not wides, and whatever its habit of growth, its origin (local or foreign), or its distribution generally. The usual type of distribution is that shown in the distribution map of *Doona* (18, p. 14), or in that of *Haastia*, &c., above.

(5) The wides of New Zealand take no notice of Cook's Strait in their distribution, while the endemics do.

(6) The maximum of the wides coincides with that of the endemics, and both decrease together from that point, the endemics much the more rapidly.

(7) The specialized (later) and highly modified genera of Podostemaceae and Tristichaceae are all strictly localized; those that resemble ordinary water plants are widely scattered, and are just as common everywhere.

To these one may add the following notes and queries, which if not successfully answered, are very fatal to the view that endemics are chiefly relicts:

(a) How, on the view that endemics are relicts, is it possible successfully to predict what has already been successfully predicted by the aid of age and area?

(b) How are the facts of the regular graduation of species, of narrowly localized endemics up, and of wides down, to be explained at all?

(c) Why is there no difference in behaviour between endemic genera and species?

(d) Why does a genus behave in just the same way in New Zealand (for example), whether endemic with small area, endemic with large, endemic in New Zealand, endemic in New Zealand and islands, endemic in New Zealand and Australia, or endemic in New Zealand and the rest of the world?

(e) Why do all endemics show graduated maps like those given above?

(f) Why are the endemics of the same order of rarity in all families and genera?

(g) Why are the endemics of the same order of rarity whether there are or are not wides in the same genera?

(h) Why is an endemic genus the rarer the more species it has (17, p. 323)?

(i) Why should the islands round New Zealand have more endemics the more wides they have (21, p. 332)?

(k) Why are the endemics of New Zealand least numerous at the ends of the islands and not in the middle, and the wides the same (20, p. 201)?

(l) Why do the endemics that reach the ends of New Zealand range on the average so much farther than those in the middle (19, p. 448)?

(m) Why are the endemics still less numerous in proportion on the islands surrounding New Zealand than on New Zealand itself, and the wides more numerous? (N.Z. Wides/Endemics 301/902, Kermadecs 45/25, Chathams 69/76, Aucklands 27/72).

(n) Why do the endemics of both northern and southern invasions (cf. this paper, below) taper down in number with the wides, but much more

rapidly, so that in the case of the southern forms they are actually less numerous than the wides in five zones?

(o) Why, if endemics are being driven in by the wides, do their areas almost invariably overlap?

(p) Why are there practically no broken areas among them?

(q) Why do the Ceylon-Peninsular India species show a range on the average intermediate between the Ceylon endemics and the wides?

(r) Why are the species endemic to New Zealand and the islands so common in New Zealand, more so than the average of the wides in that country (21, p. 331), and why are the wides that also reach the islands yet more common again? What is there in ranging to these little groups of islands to make plants so well suited to New Zealand?

(s) Why should youth make a species more capable of spreading?

(t) Why should a species get to more islands the younger it is (21, p. 330).

(u) Why do endemics occupy so much larger an area in New Zealand than in Ceylon, even proportionately to the size of the country (19, p. 454)?

(v) Why do fern endemics, which must on the average be older, show greater distribution areas than Angiosperm endemics (22, p. 340)?

(w) If the wides are the younger, there is no reason why they should be specially closely related to the endemics, and why should they show the same arithmetical relationships throughout?

(x) Why do endemics and wides, in the majority of cases, belong to the same genera?

(y) Why do the wides give flatter curves for local distribution than the endemics?

(z) Why are the endemics so often on mountain-tops?

(aa) Why do separate species of endemics occur for different mountains near together (16, p. 132)?

(bb) Why do the endemics belong almost entirely to widely spread and successful genera, and this even more on the very isolated islands like the Chathams?<sup>1</sup>

(cc) Why does the area covered in New Zealand go, not with that covered in the world in general, but with that covered in what I have called the New Zealand archipelago (21, p. 331)? This seems to me to exclude both Natural Selection and absolute youth.

<sup>1</sup> The Chatham endemics belong to *Geranium*, *Aciphylla*, *Pseudopanax*, *Corokia*, *Coprosma*, *Olearia*, *Cotula*, *Senecio*, *Sonchus*, *Cyathodes*, *Myrsine*, *Gentiana*, *Veronica*, *Carex*, *Poa*, *Festuca*. The Auckland endemics belong to *Ranunculus*, *Stellaria*, *Colobanthus*, *Geum*, *Azorella*, *Ligusticum*, *Coprosma*, *Olearia*, *Celmisia*, *Cotula*, *Abrotanella*, *Gentiana*, *Veronica*, *Plantago*, *Urtica*, *Bubinella*, *Hierochloe*, *Deschampsia*, *Poa*.

### THE RELATIVE AGE OF TREES, SHRUBS, AND HERBS, AND THE SOURCES OF THE NEW ZEALAND FLORA.

Relative age is a very large question indeed, and I may be pardoned if I point out that for the present, at any rate, age and area is quite incompetent to solve it, though Prof. Sinnott so far extends the application of my hypothesis as to include that point among its possibilities. I have nowhere committed myself, so far as I can find, to an expression of opinion upon this question. These groups are ecological, not systematic, and nothing is more clear than that they are extremely polyphyletic, a fact which makes determination of relative age difficult. There are very few families composed entirely of trees or herbs, and even among genera there are many containing two or more forms. In Ceylon 87 show this, in New Zealand 12 : these include such well-known genera as *Polygala*, *Hypericum* (in both countries), *Abutilon*, *Hibiscus* (in both), *Helichrysum*, *Senecio* (in both), *Dracophyllum*; *Solanum* (in both), *Euphorbia*, *Phyllanthus*, *Ficus*, *Urtica*, and many more. Even in such a markedly herbaceous family as Cyperaceae there is one tree, occurring in West Africa. Does Dr. Sinnott regard this as a solitary prehistoric Cyperacea now dying out?

Except in the case of genera, whose members are systematically allied, and where (17, p. 337) it appeared to me probable that their distribution 'in wheels within wheels', exactly like the species in any one country, was easily explicable on the hypothesis of age and area, I have only applied this hypothesis to groups of twenty allied forms within a given country. It might perhaps be possible to prove, by careful application of the hypothesis, that within New Zealand trees were older than herbs, but that would prove nothing as to the absolute age of either group, except that the trees were the first comers. Only if it could be shown that they were the first comers to most countries would it be possible to say that they were in reality the older group.

I have nowhere committed myself, so far as I can find, to the view that all plants occupying equal areas are of the same age, though I maintain that all are governed alike by the law of age and area. I have not attempted to decide, for instance, which of two genera or species, one a tree, the other a herb, is the older, when both occupy the same area in the same country. But in any case my figures show that each group is ruled by age and area, and a Dipterocarp (tree) with a radius of 200 miles in all probability bears the same relationship in age to one with a radius of 100 miles as does a Composite (herb) with a radius of 200 to one with one of 100. *A priori* it would seem probable that the herb would spread the more rapidly, but one must remember another complication, that some herbs are of open

ground, and cannot spread in forest, others are forest herbs and cannot spread in open ground.

Dr. Sinnott argues as if herbs could of themselves, without outside assistance of some kind, supersede and replace forest. There is little evidence for this, though the fact that trees can replace herbs is familiar to every one who has lived in the midst of forest vegetation. So far as I am aware, some extraneous assistance is needed for the reverse to happen, such for instance as the operations of man, or a desiccation of the climate.

So far as age and area is concerned, trees, shrubs, and herbs all behave in exactly the same manner. Except in the position of the maximum there is no difference to be seen in the figures quoted for the genera in Tables V and VI (19, p. 446). All show a gradual increase to a maximum, usually in the South Island, and a falling away again, or an increase to a maximum at the north, as in *Pittosporum*. Yet *Clematis* is shrubby, *Ranunculus* and *Lepidium* herbaceous, *Pittosporum* is composed of trees and shrubs, *Carmichaelia* is shrubby, *Tillaea* herbaceous, and so on.

Another point that Dr. Sinnott is apt to forget is that trees, shrubs, and herbs may come to a country from different sources, so that the tracing of their relative ages is rendered still more difficult. In this connexion it is worth while to see what can be learnt about New Zealand from a consideration of Tables IV, V, VI of my paper (19). Though the discovery of the facts contained in these tables was the result of a prophecy made by aid of age and area, the tables themselves contain nothing but bald facts. One of the first points that one notes is that whilst the majority of the families (and genera) show figures leading up to a maximum in the south, and falling away again (and that quite regularly for every family and genus in the flora), a very fair number, e.g. Pittosporaceae or Myrtaceae, commence with their maximum to the north, and taper away towards the south. Whatever be one's views as to age and area, it is quite clear from the tables that the previous distributional history of these families was different from that of the others. Table IV gives only endemic species, but if we add to them all the families with northern maxima, we get the result shown in Table I, below.

The first glance shows that all these families are markedly Indo-Malayan, though they contain in New Zealand a few genera whose southern location indicates a southern derivation, and indeed some of them, like *Laurelia* in Monimiaceae and *Donatia* in Saxifragaceae, are South American genera. But the overwhelming majority are Indo-Malayan.

TABLE I.

Family.	Shrubs and Trees.			Herbs.		
	Wide.	N.Z. and Islands.	N.Z. only.	Wide.	N.Z. and Islands.	N.Z. only.
1. Pittosporaceae	—	1 (K.)	18	—	—	—
2. Rutaceae	—	1 (K.)	2	—	—	—
3. Meliaceae	—	—	1	—	—	—
4. Olacineae	—	—	1	—	—	—
5. Rhamnaceae	3 (1 Ch.)	—	2	—	—	—
6. Sapindaceae	1	—	1	—	—	—
7. Anardiaceae	—	1 (K., Ch.)	—	—	—	—
8. Saxifragaceae	—	—	7	1 <sup>1</sup>	—	—
9. Myrtaceae	1 (Ch.)	1 (Au., Cpl.)	16	—	—	—
10. Passifloraceae	—	—	1	—	—	—
11. Cucurbitaceae	—	—	—	1 (K.)	—	—
12. Araliaceae	—	2 (K., Au.)	12	—	1 (Snares)	—
13. Cornaceae	—	—	4	—	—	—
14. Caprifoliaceae	—	—	4	—	—	—
15. Sapotaceae	1	—	—	—	—	—
16. Oleaceae	—	—	4	—	—	—
17. Apocynaceae	—	—	2	—	—	—
18. Lentibulariaceae	—	—	—	1 <sup>2</sup>	—	5
19. Gesneriaceae	—	—	1	—	—	—
20. Myoporaceae	—	1 (K., Ch.)	—	—	—	—
21. Verbenaceae	1	—	2	—	—	—
22. Nyctaginaceae	1	—	—	—	—	—
23. Amarantaceae	—	—	—	1	—	—
24. Piperaceae	1 (K. Ch.)	—	—	2 (1K.)	—	—
25. Monimiaceae	—	—	2	—	—	—
26. Lauraceae	—	—	3	1 <sup>3</sup>	—	—
27. Proteaceae	—	—	2	—	—	—
28. Santalaceae?	—	—	2	—	—	—
29. Balanophoraceae	—	—	—	—	—	—
30. Urticaceae	—	1 (Ch., Au., Ant.)	4	3 (1K.)	—	1
31. Palmae	1 (Ch.)	—	—	—	—	—
32. Pandanaceae	—	—	1	—	—	—
33. Typhaceae	—	—	—	2 (1K.)	—	—
Total	10	8	92	12	1	7

The islands reached by some of the species (K., Ch., Au., &c.) are shown in brackets.

To these may probably be added Magnoliaceae, Ficoideae, Convolvulaceae, Solanaceae, and Chenopodiaceae, as well as parts of such families as Malvaceae, Tiliaceae, Orchidaceae, Liliaceae, Cyperaceae, Gramineae, &c., and many single genera in other families.

From this table one may at once draw an important conclusion. If one mark the zones in which the different species occur, one gets the result:

TABLE II.

	1-100 m.	101-200 m.	201-300 m.	301-400 m.	401-500 m.	501-600 m.	601-700 m.	701-800 m.	801-900 m.	901-1000 m.	1001-1080 m.
Wides	13	11	12	10	8	8	7	7	6	5	3
Endem.	84	89	87	78	64	62	50	40	35	32	14

<sup>1</sup> *Donatia Novae Zelandiae*: range 480-1080 m. and Tasmania; S. American genus.

<sup>2</sup> *Utricularia monanthos*: range 380-1080 m. and Tasmania; probably arrived from south.

<sup>3</sup> *Cassytha paniculata*, perhaps an introduction, but cf. Guppy, *Nat. in Pacific*, p. 56, &c.

The falling off from north to south is extremely regular. If we plot these figures in a curve we get the result shown in Fig. 6. Both fall off from north to south, but the endemics fall off much more rapidly; both have maxima and minima at the same points, or practically the same. Nothing as yet proposed but age and area will explain such curves as these. The wides are older, and have mostly spread so far down New Zealand that their curve is nearly flat: the endemics are younger, and have only spread to varying distances down the islands, so that their curve rapidly falls off. In face of a curve like this one cannot maintain that the wides are killing out the endemics.

Now if these families had arrived in New Zealand by casual transport across the sea, it is very difficult to believe that their arrangement would

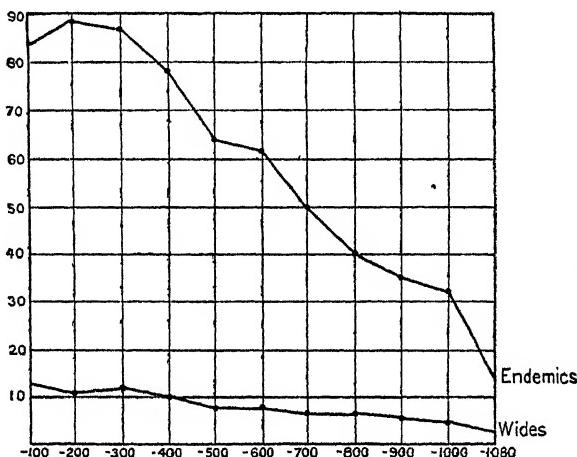
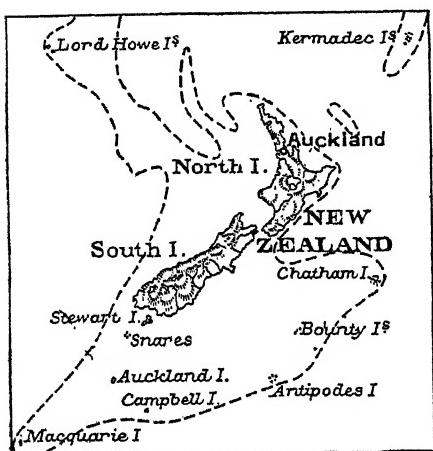


FIG. 6.

have shown such symmetry. It would seem much more probable that they arrived at some comparatively narrow point of entry in the north. The diagram on p. 442 (19) gives an idea of what may happen under age and area, and shows that the maximum of endemics is to be expected at or near the point of entry. In connexion with this diagram, as Dr. Sinnott and others say that my whole argument about New Zealand hinges on the fact that I commence with an hypothetical entrance of the flora at the centre, it may be worth while to point out that the result will be similar wherever be the point of entry, the maximum of the endemics always being near to that point. Further, it is not absolutely necessary that it be a *point* of entry; the result would be similar if it were a belt of entry. If, for example, the entry were by the whole belt from 300 to 700 miles, above E 2 and 3, we should get a zoning

The curves for the species of this northern invasion give one to imagine that the point of entry was comparatively narrow, perhaps not very much wider than one or two of the zones of 100 miles into which I have divided the islands. As New Zealand is mountainous throughout, it is possible that birds, which would be likely usually to land in the same hills, might have brought the plants, but many of them have seeds very much unsuited to bird transport. It would therefore seem probable that they must have arrived by some *land* bridge, and the same applies to the remainder of the flora, which shows a southern maximum. This is not denying that casual transport may occur, and indeed there are cases, like *Ipomoea palmata* on the northern coast, whose limited or peculiar distribution is more readily explained in this way than by a land bridge. As after 300 miles from North Cape both wides and endemics begin markedly to fall off, it would appear probable that the bridge reached New Zealand somewhere within the first 300, or at most 400 miles from the north.

The next question naturally is, was it a bridge by way of the Kermadec Islands (see map)? When one comes to look into this, one finds that only 11 genera (representing 10 families) out of 60 in these 33 families occur in the Kermadecs, and of these it is practically certain that some, e.g. *Sicyos*, are genera which really entered by way of those islands.<sup>1</sup> It would therefore seem probable that



New Zealand and outlying islands. The dotted line is the 1,000 fathom limit.

the connexion by which these families entered was not that way. But as they are all represented in tropical Australia, which is part of Indo-Malaya, it would seem likely that one of the two strips of shallow water shown in the map as running down from NE. Australia represents the remains of the bridge by whose means they arrived.

Passing on now to the constitutional habit of these plants, which is given in the table, one notices at once that they are nearly all shrubs and trees, only 20 out of 130 being herbs. The proportion of trees and shrubs is no less than 84 per cent. And of the 110 no fewer than 46 are trees, out of a total of 72 trees in New Zealand. If we add the other families of probable northern origin, we get a total of 48 trees. In any case it is clear that the bulk of the trees in New Zealand arrived from the north, or were evolved from or in genera that arrived in this way.

<sup>1</sup> The question of the union of New Zealand and the islands will be discussed in later papers.

When we examine the zoning of these species, given above, it is clear that on the whole they are very old in New Zealand, for they take no notice of Cook's Strait, between the fifth and sixth figures, and comparatively little of Foveaux Strait, between the tenth and eleventh. But this is no proof, without much confirmatory evidence from other countries, that they are absolutely very old.

If from the list of families we exclude these which have just been considered, it will be noticed that all the rest have their maximum of species to the south, sometimes towards the north end, sometimes towards the south end, of the South Island. If we take only those families which have a maximum in the zone 701-800 miles<sup>1</sup> (south of North Cape), or to the south of that, we get the following list of families :

TABLE III.

Family.	Shrubs and Trees.			Herbs.		
	Wide.	End. N.Z. and N.Z. only. Islands.	Wide.	N.Z. and Islands.	N.Z. only.	
1. Ranunculaceae	—	—	9 <sup>2</sup>	6	—	30
2. Cruciferae	—	—	—	4	2	16
3. Caryophyllaceae	—	—	—	5	2	8
4. Portulacaceae	—	—	—	2	—	1
5. Rosaceae	—	—	4 <sup>3</sup>	5	—	7
6. Droseraceae	—	—	—	5	1	—
7. Haloragidaceae	—	—	—	9	1	9
8. Onagraceae	—	—	3 <sup>4</sup>	4	7	17
9. Umbelliferae	—	—	—	10	1	43
10. Stylidiaceae	—	—	—	—	1	6
11. Campanulaceae	—	—	—	2	2	7
12. Gentianaceae	—	—	—	2	—	13
13. Scrophulariaceae	1	1	65 <sup>5</sup>	9	—	33
14. Plantaginaceae	—	—	—	—	1	5
15. Juncaceae	—	—	—	15	2	7
16. Naiadaceae	—	—	—	12	—	1
17. Centrolepidaceae	—	—	—	1	2	3
18. Gramineae	—	—	—	35	9	53
Total	1	1	81	126	31	259

Now if we zone these families from north to south; as we did with the northern group, we get the result :

TABLE IV.

	1-100 m.	101-200 m.	201-300 m.	301-400 m.	401-500 m.	501-600 m.	601-700 m.	701-800 m.	801-900 m.	901-1000 m.	1001-1080 m.
Wides	83	87	100	97	101	105	104	102	100	91	51
End., N.Z. and Islands	16	17	19	21	24	27	28	28	29	29	18
End., N.Z.	33	55	76	95	115	189	205	220	228	166	46

Both wides and endemics taper off from south to north, but, as in the case of the northern families, the endemics taper down far more rapidly.

<sup>1</sup> The 800-mile line passes a trifle south of Raleigh and Timaru.<sup>2</sup> Clematis.<sup>3</sup> Rubus.<sup>4</sup> Fuchsia.<sup>5</sup> Veronica.

The tapering is especially shown in the last 400 miles at the northern end of New Zealand, where the endemics actually fall below the wides. When plotted into curves, as in Fig. 7, it is clear that these families exactly reverse the behaviour of the northern families just dealt with.

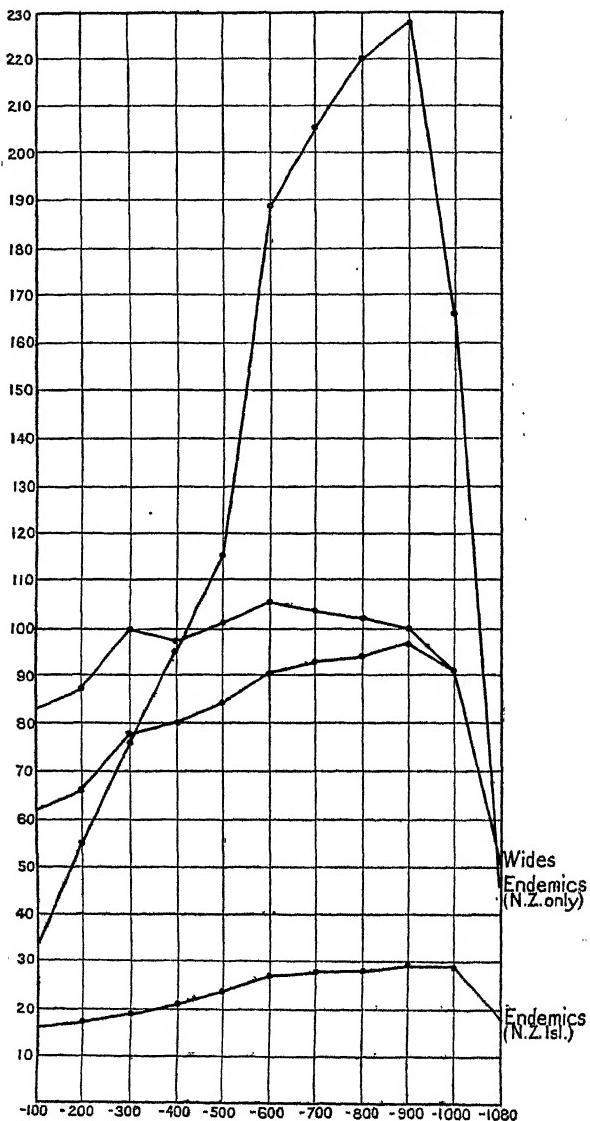


FIG. 7.

In Gramineae and one or two other families, there are quite a number of wides beginning at the North Cape and ending somewhere to the south, e.g. *Paspalum scrobiculatum* ranges as far as East Cape, *P. Digitaria* as far

as Coromandel, *P. distichum* as far as Waikato River, all at distances not over 430 miles from North Cape. It is clear that these are northern types, and that some of these families invaded New Zealand at both ends. If we subtract them, we get for the wides the result:

less	83	87	100	97	101	105	104	102	100	91	51
	21	21	23	17	17	14	11	8	3	—	—
	62	66	77	80	84	91	93	94	97	91	51

a result which brings the wides exactly into line with the endemics, and with the maximum at the same zone (see lower of two curves for wides).

Many will perhaps object to my treatment of these families, and say that if one pick out the families with a maximum at a certain point, and add them up, it is not surprising if they show great regularity in the curve. True, but the surprising thing has already been pointed out (19, p. 444), in giving these curves for the individual families, that *all* of them show such curves, and similar curves, rising to a maximum and then falling off again—a result quite unsuspected until I had applied to the New Zealand flora the ideas suggested by age and area, and had added up for each zone the actual plants that occurred therein.

It seems probable that these families, or a great part of them, which show as great symmetry in their curves as did the northern families, arrived in New Zealand by a southern route, which perhaps reached New Zealand with its central part about the middle of the southern half of the South Island. The maxima are at 800–900 miles, or a little north of Dunedin, which lies on the 900-mile line. Whence this southern bridge came is a more complex question that must be left for the present unanswered.

Passing on now to deal with the constitutional habit of these plants, the first thing that one notices is that not only are they much more numerous (though of fewer families) than the northern invasion, but they are nearly all herbs, there being only 83 shrubs and no trees in 499 species. Of the shrubs 67 are Veronicas. The proportion of herbs is 83 per cent., almost exactly equal to the percentage of trees and shrubs in the northern families that we first dealt with. Now as these herbs are mostly types of the Northern Hemisphere, it is clear that herbs must be very ancient; but as to whether they are older or younger than the trees and shrubs, the flora of New Zealand gives little clue. On the whole, one imagines, these southern types perhaps arrived in New Zealand later than the northern, for they do take some notice of Cook's Strait, quite a number being held up there, and they take about as much notice of Foveaux Strait as the northern forms, though they must have started so much nearer to it. Their curve of zoning of endemics, also, is rather more sharp, which would tend to show that the endemics were younger than the northern endemics. But it is clear that New Zealand alone will not permit of drawing any conclusion with the aid of age and area as to the relative ages of the different habits of growth.

Of the remaining families of the New Zealand flora, nine at least are composed of so few species, and range so uniformly along the whole length of the islands, that age and area will not permit of any deductions as to their source. There thus remain twenty-six families. Of these several, e.g. Orchidaceae, can be easily separated into two well-marked groups, one commencing at the North Cape and ranging to a greater or less distance southwards in New Zealand, and the other commencing at the south end and ranging northwards, so that it seems justifiable to infer that these families have invaded New Zealand both from the north and from the south. But when these have been taken out, there still remain a few families like Stackhousiaceae, Epacridaceae, and Myrsinaceae (Australian families) which have a fairly marked maximum in the middle of New Zealand, and range from that to the north and to the south. There is little or no evidence to show by which (if either) of the two routes already discussed these plants arrived.

From what has been said above, it will be clear that whilst the application of age and area to the problem may lead to fairly good evidence as to what has happened in the past in New Zealand itself, the hypothesis cannot be used in its present early stages to give evidence for or against the question of the relative age of trees, shrubs, and herbs. This question is really a very large one, and rendered much more complex by such questions as polyphyletic origin, &c.

#### AGE TENDS TO EXTINGUISH OLD SPECIES?

Like the question of the greater age of woody vegetation, this is a very large problem, and I shall simply endeavour to show that it is a somewhat complex one, not easily to be solved off-hand. Dr. Sinnott proposes an hypothesis to the effect that 'the longer a successfully invading species remains in an isolated area . . . the less common it tends to become until it is actually "swamped" out of existence—quite the reverse of the "age and area" idea'. He suggests that 'some may simply be exterminated outright, and some by continual crossing with new forms may ultimately lose their specific identity'.

There is no doubt that the fact that genera are common in these floras with endemics only, and no wides, is a feature which requires explanation; but as the genera with endemics only behave exactly like those which also contain wides, or like the endemic genera, the fact that it cannot at the moment be explained does not in the least militate against the hypothesis of age and area. Age and area may not agree with other views as to this or that, but it must be remembered that it is based upon very clear and definite figures, which must either be controverted or explained in some other way—they are far too striking to go without any explanation. It is somewhat difficult to controvert figures which simply represent bald facts, and if age and area be not accepted, it is consequently necessary to have

some other hypothesis, which must be mechanical, owing to the fact that the figures show such mechanical regularity.

Dr. Sinnott bases his views largely on the undoubted fact that the proportion of 'swamped' genera is larger in the more outlying of the big islands—in New Zealand than in Ceylon, in the Hawaiian islands than in New Zealand. But that mere isolation is not sufficient as an explanation would seem to show in the fact that in the very isolated islands round New Zealand the proportion is not so high as in New Zealand itself. In New Zealand 151 genera out of 316 show it, in the Kermadecs only 8 out of 62, in the Chathams the same, and in the Aucklands 12 out of 64. In none of the islands is the proportion anything like so high as in New Zealand, and it is highest in the Aucklands, which were probably nearest to the incoming stream of plants. On the other hand, the number of genera which are swamped *in New Zealand* is 13 in the Kermadecs, 33 in the Chathams (the most isolated), and 26 in the Aucklands, facts tending to show that the swamped genera were in existence fairly early opposite to the Chathams, and therefore were rather old in comparison to some of the rest, though even in the Chathams the unswamped genera are almost as numerous (29).

Another test that we may apply is to find the proportion of 'swamped' genera in the northern and southern invasions of plants above discussed. The northern shows 45 out of 75 or 60 per cent., while the southern shows 36 out of 108 or 33 per cent. We have seen that probability is in favour of the greater age in New Zealand of the northern invasion, so that to some extent this speaks in favour of Dr. Sinnott's views, in a general and purely local sense. But as only one herb (*Elatostema*) is swamped in the northern invasion, and all the shrubs but one (*Veronica*) in the southern, it is, it seems to me, equally possible that swamping may go with woody habit, and further tests are necessary.

Of the 151 'swamped' genera, 45 Dicotyledons and 30 Monocotyledons are herbs, or 50 per cent., while of the 165 unswamped, 75 Dicotyledons and 63 Monocotyledons are herbs, or 83 per cent. Of these unswamped genera 99 have no endemics, and of these 85, or 85 per cent., are herbs, while of the 66 with endemics 53, or 80 per cent., are herbs. From these figures it would seem that the evidence is just as good for the connexion of swamping and woody nature as of swamping and age.

The Coniferae are probably older than the flowering plants, and as they have no wides at all in New Zealand, this speaks in favour of age, but they are also all woody plants. The Ferns, on the other hand, which are probably older again, show very little 'swamping'; only 5 genera out of 31 exhibiting this phenomenon. Of these it may be noted that three are the only tree-ferns in New Zealand. The remaining two, and all the unswamped genera, are herbaceous. It is evident that the question of swamping must be first disentangled from the question of the relatively greater

age of woody vegetation, and that, as in that case, the investigation of any one or two floras is quite insufficient to provide a solution.

If, as Dr. Sinnott suggests, the absence of wides in a genus had anything to do with the age of the genus, one would rather expect to see some difference in the figures of distribution of the two classes, especially in the zonal figures, in which Cook's Strait may interfere with the younger forms. But in actual fact the figures for the two classes show the most extraordinary similarity :

TABLE V.

Southern genera.												
Swamped	83	110	139	158	179	236	240	242	232	186	152	75
Not	45	64	82	99	112	188	193	202	189	189	152	48
Northern genera.												
Swamped	77	78	76	72	58	56	50	37	32	31	12	12
Not	11	11	11	7	7	7	6	6	4	3	3	3

The parallelism is most remarkable, and both groups show the holding up at Cook's Strait almost equally in the southern genera, and not at all in the northern.

A very interesting comparison, which does not harmonize very well with the hypothesis of swamping, may be made among the various classes of wides. There are 66 genera containing both wides and endemics, 99 with wides only. Of the 66, 35 genera have a second wide species, and 22 more than one, and of the 99, 24 genera have at least one extra species, and 5 more than one. This alone would go to show that it was mainly the older wides which gave rise to the endemics. But now if we pick out first the commonest (most widespread) wide in each genus, next the second commonest (for the 35), and the others (for the 22), but, as the numbers are small, lump together all the others for the 24 genera with no endemics and more than one wide, we get :

TABLE VI.

Class.	Wides with endemics.			Wides without endemics.	
	First.	Second.	Others.	First.	Others.
1	37 (56 %)	13 (37 %)	7 (10 %)	21 (21 %)	4 (12 %)
2	9	5	9	27	13
3	3	3	14	9	—
4	4	4	11	8	2
5	5	1	6	8	1
6	3	3	4	9	—
7	1	2	7	7	2
8	1	5	3	5	3
9	—	—	3	5	2
10	3	1	5	—	5
	66	35	69	99	32
Rarity	2·5	3·4	4·5	3·6	4·6

It will be seen at a glance that the commonest wides in the genera with endemics are much more widespread than those in the genera without,

ranging on the average 132 more miles (difference 1·1, each 0·1 representing 12 miles).

When one comes to examine these figures a little more closely, one notices in the first column that 37 wide species (56 per cent.) cross Foveaux Strait, ranging the entire length of New Zealand, while only 9 range the two main islands without crossing the Strait. In the second column only 37 per cent. cross the Strait, and in the third only 10 per cent. This is as one would expect from species picked in order of commonness. But when one goes on to the wide species without endemics, one finds that even the first column shows only 21 per cent. crossing the Strait, and 27 per cent. held up there. It is clear that on the average these wide species arrived in New Zealand as late as, or rather later than, the second wide species in the genera which have endemics, and in the same way their later species (column 5), though 19 out of 24 are second arrivals, are rather later than the third (and later) arrivals of the first lot of wide species, those with endemics. This table shows, in a very clear manner, that the wide species with endemics are on the whole earlier arrivals than those without.

One may examine these tables in another way. The 46 wide genera with endemics that occur in classes 1 and 2 have altogether 304 endemics, those in classes 3, 4, 5, and 6 have 148,<sup>1</sup> and those in classes 7, 8, 9, and 10 have 7 only; again age is indicated as being more likely to 'involve' endemics. The 151 swamped genera have between them only 481 endemics, against 421 for those 66 genera which also contain wide species. These facts go to show that on the whole it is the older wide species which are accompanied by endemics, not the younger, as might be expected upon the hypothesis of swamping.

Dr. Sinnott's objection that age and area will not explain the New Zealand flora because it is based (cf. my diagram in 19, p. 442) on a central point of arrival of the flora, and it is generally agreed that the flora arrived in two or more directions, seems to me due to misunderstanding of my tables, which show with great clearness that there were at least two sources. I have already been<sup>1</sup> into this question, but may just call attention to the fact that though there is a very clear northern invasion, the southern is so much larger that the total figures show practically the same result as the southern only—the northern are lost in them.

I am much indebted to my daughter Margaret for drawing the diagrams here reproduced, and to Dr. H. B. Guppy, F.R.S., for criticisms.

<sup>1</sup> Including 77 Veronicas and 22 Myosotises.

## SUMMARY.

The paper is a reply to criticism, and brings up new facts about the distribution of plants in New Zealand. It is shown that age and area now occupies a strong position, because by its aid numerous prophecies as to geographical distribution of plants have already been made, and have proved to be correct upon examination of the facts.

Incidentally the flora of introductions into Ceylon is analysed, and it is shown that there is practically no evidence of large spread without the aid of man.

To make more clear the true meaning of the tables of figures that have been published, diagrams are given, showing the range in New Zealand of the species of *Ranunculus* and other genera. Their resemblance to the rings made by throwing a stone into a pool will at once be noticed, and is a strong argument against any but a mechanical explanation of these ranges. The widest range farthest, the endemics successively less.

A section is devoted to the activity of other factors than age, which have already been considered in detail in other papers. The question whether endemics are chiefly relicts is then discussed, and it is shown that for the vast majority the evidence is much against such being the case. Explanation by age and area is simpler and more convincing. Natural Selection cannot explain such cases as *Ranunculus Lyallii*, which are very numerous. Finally, there is given a list of twenty-eight awkward questions for the supporters of the dying-out hypothesis, questions which if not successfully answered are very damaging to that hypothesis.

The relative age of herbs, shrubs, and trees is then dealt with by showing that this question is really very complex, and at present far beyond the capacity of age and area to answer. The figures already given for distribution in New Zealand are analysed, and it is shown that thirty-three or more families have their maximum at the far north, and taper down steadily to the south. This goes to show that there must probably have been a northern land bridge reaching New Zealand from some part of Indo-Malaya (probably N. Australia), and similarly there are eighteen or more families which must probably have reached New Zealand by a southern bridge from some region abroad. The northern families are mainly trees and shrubs, the southern herbs. This alone shows how difficult is the question of relative age, and it is also pointed out that I have not claimed the same age for two plants occupying the same area, unless systematically related. Nor, it seems to me, are age and area and greater age of trees incompatible hypotheses.

Dr. Sinnott's hypothesis of swamping is considered, and it is shown that while it may have certain probabilities in its favour, the evidence is very conflicting. It is more common on the whole in genera of very ancient arrival in New Zealand, but it is also much more common in trees and

shrubs than in herbs. 'Swamped' and unswamped genera behave alike in their distribution through New Zealand. Swamping is rare in the Ferns, which on the whole must be old.

Finally, it is shown that the widest with endemics are on the whole probably older than those without.

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# On the Branching of the Zygopteridean Leaf, and its Relation to the probable 'Pinna' Nature of *Gyropteris sinuosa*, Goepert.

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With three Figures in the Text.

## I. THE BRANCHING OF THE ZYGOPTERIDEAN LEAF.

IN 1909 Dr. Paul Bertrand (1) published an exhaustive comparative account of the Zygopteridean leaf, exhibiting in great detail the complex forms assumed by the vascular strands. A special feature of the work was the attention paid to the branching of the vascular axis of the leaf, to supply the secondary and tertiary axes ('pinnae' and 'pinnules'). Where possible the further branching of the tertiary axes was also described, and the vascular supply to the aphlebiae was traced.

It was Dr. Bertrand's detailed account of *Stauropteris oldhamia* which allowed the structure of this till then rather puzzling species to be interpreted in terms of the typical Zygopterid leaf-trace. Assuming that the largest axis known is the primary rachis, it is described as bearing altogether four series of pinnae (secondary raches), in pairs alternating on the right and left sides of the leaf. The vascular supply to these branches is as follows: From the cruciate xylem-mass of the primary rachis (Fig. 2, D) a single large strand, elongated in the antero-posterior plane, becomes detached laterally. This is the *pièce sortante* of Bertrand. It never appears to acquire a cortical sheath of its own, for it at once divides, symmetrically along the right and left plane, into two halves, the *demi-pièces sortantes* of Bertrand. Each of these is described as entering one of the two so-called 'pinnae' (secondary raches) on that side.<sup>1</sup>

If the branching primary axis of *Stauropteris* were viewed superficially, without regard to the vascular anatomy, it would be perfectly natural to regard the lateral axes as 'secondary' raches. But in the presence of the single embedded strand (*pièce sortante*) which comes off from the side of the primary petiolar strand, and which by subsequent division gives rise to the

<sup>1</sup> These latter in their turn branch in an almost identical fashion, and the process is repeated in several generations of axes; but we need not follow this in detail.

two strands supplying these axes, the latter cannot, I think, be regarded as morphologically equivalent to secondary rachis ('pinnae'). There appears to be no alternative but to regard them as *tertiary* rachis ('pinnules'), and the large strand ('*pièce sortante*') still enclosed in the cortex of the primary axis as the strand of a *secondary* rachis ('pinna') which has not become free. See Fig. 1.

Stress has been laid on this apparently unimportant point, for the reason that it has been responsible for a misunderstanding on one point of some theoretical importance: the mode of branching of the frond in some Zygopterideae has been considered

to be unique among vascular plants: fronds have been described as bearing *four* series of pinnae, two on each side of the primary rachis. In some inexplicable way, the morphological significance of the embedded pinna-trace has hitherto escaped the notice of most authors, not only in *Stauropteris*, but also in *Dineuron*, *Eatapteris*, *Metaclepsydopsis*, and *Diplolabis*. In the two last-named genera Dr. Gordon (5) calls the embedded trace the 'pinna-trace-bar' (p. 716, foot-note) and describes it as subsequently dividing into two 'pinna-traces'. In *Eatapteris* also, where the homologue of the

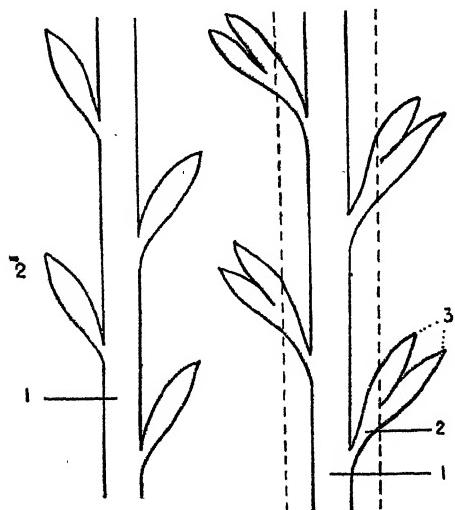


FIG. 1.

embedded trace is formed by the fusion of two originally separate pieces, neither Bertrand nor Kidston and Gwynne-Vaughan (9) have given it the interpretation which appears to be the only admissible one. Dr. Kidston, however, in 1908 (7) correctly regards this strand in *Dineuron ellipticum*, Kidst., and *Metaclepsydopsis duplex* as a pinna-trace. But the description by Kidston and Gwynne-Vaughan (1910) does not differ in this respect from Bertrand's interpretation.

The condition in *Zygopteris primaria*, Cotta, unfortunately remains obscure. According to Bertrand (1909, p. 137) it is not possible to say whether the pinna-trace is formed as in *Eatapteris* by the fusion of two originally distinct pieces, or whether it comes off as a single arc and is thus more closely comparable to the condition in *Diplolabis*, *Metaclepsydopsis*, and *Dineuron*.<sup>1</sup>

The misconception, now for the first time cleared up, apparently dates

<sup>1</sup> The latter would appear to be more probably the case, for *Z. primaria* and *Diplolabis* have

as far back as 1874, when Williamson (19) wrote as follows in reference to the branching of the vascular axis of *Rachiopteris (M.) duplex*: ‘I know of no recent fern in which the secondary branches [meaning pinnae] of the petiole are thus given off in pairs, which pairs are distichously arranged on the primary axis, and each of which secondary petioles sustains ternary ones arranged distichously. Not only will a similar case come before us on a later page of this Memoir, in Corda’s genus *Zygopteris*, but the structure of the *Rachiopteris oldhamia* just described suggests the possibility that a somewhat similar arrangement may have existed in its case.’ Stenzel, writing in 1889 (17), divided the genus *Zygopteris* into two main sections (*Zygopteris* proper and *Ankyropteris*) according to the biserrate or quadriserrate arrangement of branches which he erroneously regarded as being *all secondary raches*. Solms-Laubach (15), while justly criticizing Stenzel’s classification as being inconsistent on other grounds, passes over the first error unnoticed. Finally, Dr. Bertrand (2), throughout his 1912 paper in the ‘Progressus’, does not depart in this respect from the position he adopted in 1909, except that on p. 221 he suggests that the presence of four rows of appendages in *Diplolabis* is due to a precocious dichotomy (*dichotomie hâtive*) of the secondary petioles. After this statement he continues to regard the products of the dichotomy as secondary raches.

There are thus in the Zygopterideae, as in all known vascular plants with pinnate leaves, only two rows of ‘pinnae’ (secondary raches), one on each side of the leaf, and the supposed radial symmetry of the *Stauroppterid* leaf-trace is purely superficial. In some Zygopterideae, however (*Diplolabis*, &c.), the whole of the secondary rachis is fused with the primary rachis, though the vascular strands of the two are distinct, while the tertiary raches, formed by a dichotomy of the tip of the primary rachis, are free (Fig. 1). Each tertiary rachis then branches *monopodially*, giving off small strands which supply reduced lateral axes (*aphlebiae*), which may branch in their turn. The monopodial branching of the tertiary raches is thus clearly seen to be a reduced form of the dichotomous branching of the secondary raches. In other Zygopterideae (*Ankyropteris*) the secondary raches become free, and branch monopodially to give rise to reduced axes (*aphlebiae*).

The Zygopterideae (with the sole exception of *Stauroppteris*, according to Dr. Bertrand) are generally considered to be peculiar also on account of the leaf always branching in a rectangular system (*édification rectangulaire*, Bertrand), the principal plane of each branch-axis being perpendicular to that of its own mother-axis. The leaves of *Stauroppteris* and of all the higher Ferns, Gymnosperms, and Angiosperms are supposed by Bertrand to branch in a parallel system (*édification parallèle*), so that the branches of all orders face in the same direction.

very similarly shaped leaf-traces, lacking the peculiar horn-like outgrowths of the antennae characteristic of *Elatopteris*.

So far as *Stauroppteris* is concerned, its supposed *édification parallèle* is clearly the result of Dr. Bertrand's omission to take account of the 'embedded trace'. In Fig. 2, A, the rectangular system of branching is diagrammatically shown. The numbers 1, 2, 3, refer to the primary, secondary, and tertiary axes, and may be taken to correspond to the vascular strands of the leaf, pinna, and pinnule in the three types of Zygopterideae shown (B, C, D). The arrows indicate the planes of principal symmetry. In *Metaclepsydropsis* the tertiary strands, which at some distance from the base almost directly face the antero-posterior plane of the

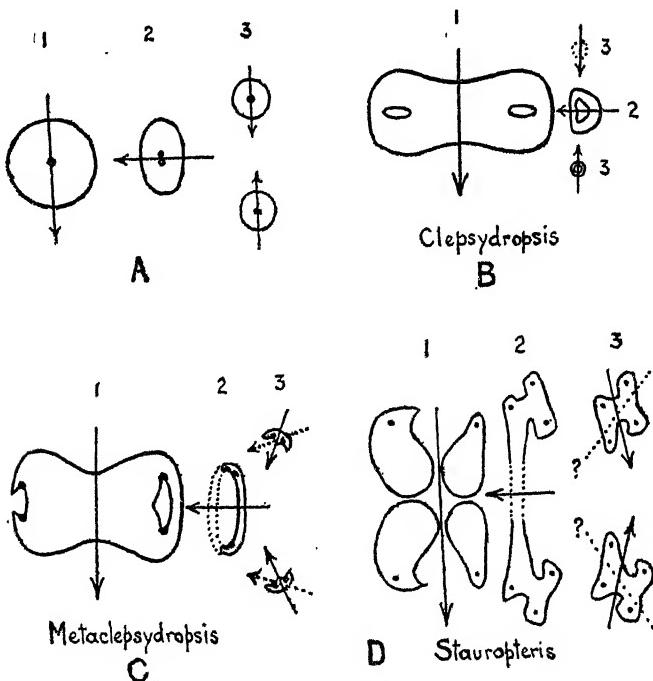


FIG. 2.

leaf (as shown by the dotted arrows), are purposely drawn almost facing the right-left plane, in order to diagrammatize. In fact, if they are traced downwards they actually do tend to assume positions nearly facing each other (see Gordon, Figs. 37–40). It is probable that their position higher up the rachis was partly responsible for the belief that they were pinna-traces directly facing the primary petiolar trace. In the case of *Stauroppteris*, however, the orientation of the corresponding strands could be verified by other means, viz. the positions of the two main phloem-masses, which, according to Bertrand, invariably lie in the principal plane on each strand.<sup>1</sup>

<sup>1</sup> I am not in a position to vouch for the correctness of the orientation of the tertiary traces as indicated in Fig. 2, D. It is possible that the arrows may have to be drawn as shown by the dotted lines. This would conform more clearly to the condition in *M. duplex* and other species.

*Stauropteris* was thus considered to possess pinna-traces facing parallel to the main petiolar trace, and an attempt was made (Bertrand, 1909, p. 177) to explain what was regarded as a surprising anomaly.

Except for *Stauropteris* our knowledge of the Zygopteridean leaf is still confined to its basal region, and it is too early to generalize as to the probable mode of branching of the distal (laminated) portions of the frond. It is to be borne in mind, however, that a number of frond genera from the same rocks as these raches still remain to be correlated, and that these fronds do not differ materially, in the orientation of their segments, from those of any living Ferns. There is thus a strong presumption that the distal (laminated) portions of the Zygopteridean leaf were in most cases held in a more or less horizontal position, all their segments being expanded in the same plane, as in the modern Ferns. The suggestion put forward by Kidston and Gwynne-Vaughan (1910, p. 474), that the peculiar habit of the Zygopteridean leaf is to be attributed to an erect position, is probably applicable only to the basal wingless portion of the rachis, except in *Stauropteris*. If, as Dr. Scott (13) has suggested, there were two kinds of leaves, sporangiferous and vegetative, the former appear to have been held strictly erect, at least in the last-named genus.

## II. ON THE PROBABLE PINNA NATURE OF *GYROPTERIS SINUOSA*, GOEPPERT.

Having arrived at the conclusion that the embedded trace of *Diplolabis* and similar Zygopterids belongs to a secondary rachis completely enclosed in the primary cortex, the question arose whether any closely allied plant existed in which the secondary rachis became free from the main cortex before dichotomizing. After this I read for the first time a description of *Gyropteris sinuosa* (P. Bertrand, 1909, p. 181) from the Carboniferous Limestone of Glätsch-Falkenberg, originally described by Goeppert (4), and was struck by the fact that the shape of its xylem portion was almost identical with that of the pinna-trace of *Diplolabis* or *Metaclepsydopsis*, the two latter being almost indistinguishable one from the other. The degree of similarity is indeed so great that it seemed probable that *G. sinuosa* is in reality a 'pinna' enclosed within its own cortex, and belonging to a genus closely allied to, if not identical with, either *Metaclepsydopsis* or *Diplolabis*. The transverse section of the vascular strand of *Gyropteris* measures about 1 cm. in length, i. e. more than four times as much as in *Metaclepsydopsis*, and if the above conjecture has any foundation, the complete plant of *Gyropteris* must be a giant among the family.

Dr. Bertrand naturally did not fail to notice the resemblance mentioned above, but he dismissed as improbable the idea that *G. sinuosa* is a secondary rachis, and provisionally regarded it as a separate genus. The

true conception of the embedded trace of *Metaclepsydropsis*, however, throws a weight of argument in favour of the conjecture, which was not available to Dr. Bertrand.

### III. DISTRIBUTION AND AFFINITIES.

The discovery in the Australian region (see below, p. 375) of a member of the Zygopterideae, a family until recently known only from Europe and West Siberia, is of considerable interest. It is one more piece of evidence in support of a fact which has repeatedly been demonstrated in the past, namely, the essential similarity of the ancient floras (and faunas) of regions of the earth now widely separated by the sea. To take the most recent case, we know that the world-wide distribution of the modern Osmundaceae has been known, within the last few decades, to have been almost paralleled by that of the fossil representatives of the family. The recent work of Dr. Kidston and the late Professor Gwynne-Vaughan has established a close relation between the fossil Osmundaceae and the Zygopterideae, and it would not be surprising if members of the latter family are discovered in other parts of the world in rocks underlying those containing Osmundaceous remains.

From anatomical evidence a common ancestry has been advocated for the Coenopterideae and the Osmundaceae (8, p. 778; 14, p. 472). It is a striking fact, however, that whereas the Coenopterideae are known to extend down to Upper Devonian times, the earliest record of the Osmundaceae is only in the Upper Permian (9, Table, p. 465). The Coenopterideae were indeed just becoming extinct when, as far as present knowledge goes, the Osmundaceae began to make their appearance. A time relation of this kind between two undoubtedly closely allied groups would seem to suggest, although somewhat vaguely, that the older group may have stood towards the younger in the relation of an ancestor. It is, however, too early to speculate on the latter view: we have not yet arrived at the limit of primitiveness which the Osmundaceae probably attained, and in the earlier rocks forms may yet be discovered which it would be difficult to assign to one or the other of these two families.

Indeed, it may be that we already have one such form in *Grammatopteris Rigolotti*, B. Ren. (12), although it has not yet been recorded from rocks older than the Permo-Carboniferous. As Kidston and Gwynne-Vaughan (8, p. 778) have remarked, this species may be a near approach to the primitive Osmundaceous type; at the same time the leaf-trace bears undoubted resemblance to that of *Dineuron*.

A few words are necessary in connexion with the affinities of some of the Zygopterideae. In classifying the different Zygopterid petioles Kidston and Gwynne-Vaughan (9, p. 470) have expressed the opinion that the most

important and far-reaching distinction is based upon the biseriate or quadriseriate arrangement of the (free) branch-axes of the leaf. In the quadriseriate forms the free branches are, as we have seen, tertiary raches formed by the terminal forking of the adnate secondary raches; in the biseriate forms the secondary raches themselves are free, and do not fork. This distinction is associated with important differences in the mode of branching of the vascular strands.

However, if there is any foundation for the suggestion that *Gyropteris sinuosa* is the free secondary rachis of a form like *Metaclepsydopsis duplex* or *Diplolabis*, a classification mainly based on the external characters would no longer be tenable. In any case it seems preferable to rely more upon the vascular structures.

The most recent scheme of phylogeny (2) is the combined result of the work of Dr. Gordon and Dr. Bertrand. In attempting to elucidate the closer relations of the genus *Stauropteris*, the latter author concludes that of all the Zygopterideae the closest resemblance is that with *Ankyropteris vibractensis*, var. *westphaliensis*, P. Bertr. (1909, p. 164). The different parts of the two leaf-traces are closely compared, and the 'filament' of *Ankyropteris* is represented as being fused indistinguishably to the side of the Stauropterid leaf-trace (see Fig. 24, p. 169). In face of the clear resemblances with *Diplolabis* and similar forms it is very doubtful that the comparison instituted by Dr. Bertrand is admissible. *Stauropteris*, like *Diplolabis*, has two planes of symmetry in the leaf-trace; the pinna-trace arises in both genera by the meeting of two processes, and in both genera it at once dichotomizes. In all these respects the leaf-trace of *Ankyropteris westphaliensis* is different: it has only one plane of symmetry; the pinna-trace arises as a closed ring, and in a manner which even for that genus is peculiar to this species; moreover, the pinna-trace does not fork.

In 1915 Mrs. E. M. Osborn (11) briefly described an important fossil from New South Wales, which, while possessing the *Ankyropteris Grayi* type of caulin stele, had leaf-traces similar to those of *Clepsydopsis antiqua*, Unger. The origin of the leaf-trace resembles that known for *A. corrugata* and—except for the absence of axillary branches—for *A. Grayi*. A short account of a similar but incomplete fossil, from a different locality in New South Wales, was read before the Cambridge Philosophical Society on February 19, 1917, and the full paper will appear in a future number of the 'Annals'. I have since learnt from Mrs. Osborn that the two plants are specifically identical.

I have no doubt that the stem-structure and leaf-trace origin in *Clepsydopsis antiqua* and *C. kirgisica* was essentially similar to that in the Australian fossil, and that the two genera *Clepsydopsis* and *Ankyropteris* should be united.<sup>1</sup> In view of this I had at first included the former genus

<sup>1</sup> A more complete discussion of this question is given in my forthcoming paper.

in the latter. Since then, however, I have had an opportunity of discussing the paper with Dr. D. H. Scott, F.R.S.. I am indeed very thankful to him for his able criticism, and for drawing my attention to the question of priority in nomenclature, which I had not fully considered. The much older genus *Clepsydopsis* (Unger, 1856) should stand, and the more recent genus *Ankyropteris* (P. Bertrand, 1909) should be merged in *Clepsydopsis*. The name *Ankyropteris* was first employed by Stenzel in 1889 (17) for one of his sections of Corda's genus *Zygopteris* (1845).

The diagnosis of the genus *Clepsydopsis* as now extended would be as follows :

*Stem* with or without 'axillary branches', or more or less equally forked. *Caulline stele*, with a solid outer xylem and a stellate 'mixed

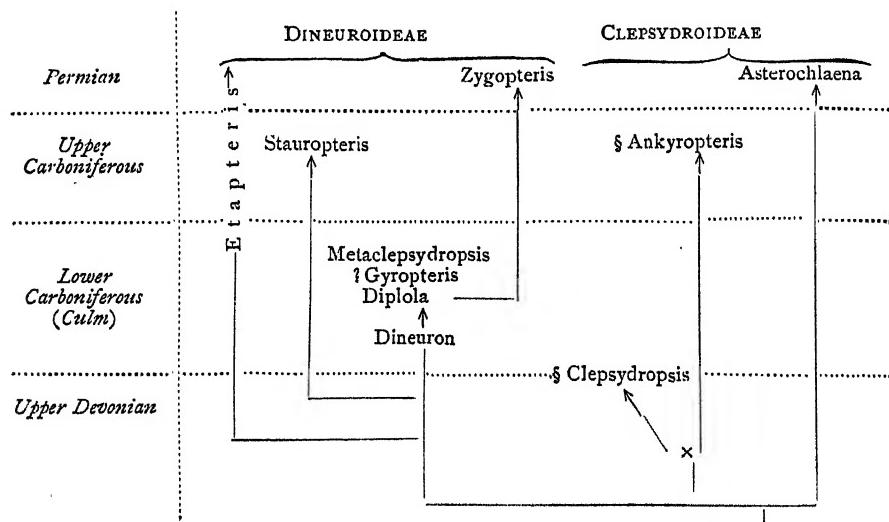


FIG. 3. Table showing inter-relations of the genera of Zygopterideae.  
Modified from P. Bertrand (1912).

pith' or internal xylem. *Leaf-traces* in  $\frac{2}{3}$  phyllotaxis, nipped off as closed rings, and distally becoming clepsydroid. *Pinna-traces* arising as closed rings and branching laterally at the base to supply similar traces to aphlebiae. Roots diarch. Known range: Upper Devonian to Upper Carboniferous (inclusive).

The known species of the genus naturally fall into two sections :

1. § *Clepsydopsis* proper.—Peripheral loops extended in the right-left plane (*C. antiqua*, Unger; *C. kirgisica*, Stenzel; and Mrs. Osborn's fossil).

2. § *Ankyropteris*.—Peripheral loops extended antero-posteriorly (including all the species hitherto recognized under the genus *Ankyropteris*, P. Bertrand).

The two sections possibly diverged from a hypothetical Devonian species of the genus (see Table, Fig. 3) which had circular loops. Kidston and Gwynne-Vaughan (9) have already commented upon the tendency in some Zygopterideae to extend their peripheral loops in the antero-posterior direction. The four Upper Carboniferous species of *Clepsydropsis*, *C. (Ankyropteris) Williamsoni*, *corrugata*, *Grayi*, *westphaliensis*, belonging to the section *Ankyropteris*, are named in order according to the degree of this extension.

*C. westphaliensis* would seem to be the most specialized; its peculiar mode of pinna-trace formation, in which process the 'filament' is reinforced by the 'antenna', is probably the result of the feebleness of the 'filament' to nip off a closed ring on its own account.

I am indebted to Dr. Scott for criticism on another point which I had omitted to discuss for considerations of space. As is well known, the pinna-trace in the genera *Metaclepsydropsis*, *Diplolabis*, *Stauropteris*, and *Dineuron* comes off as a single piece, while in *Eapteris* it arises in two distinct pieces which soon unite. It might at first seem doubtful, as Dr. Scott pointed out to me, that there is in *Eapteris* also a single row of pinna-traces on each side of the leaf-trace. This objection is adequately met by saying, as Dr. Gordon has already done (6, p. 186), that *Eapteris* has a more highly specialized form of pinna-trace origin than the other four genera, with which (see above, p. 370, foot-note) probably *Zygopteris primaria* will also have to be reckoned. In this respect *Eapteris* would thus stand towards the other genera of Zygopterideae as many higher Ferns with a double leaf-trace stand towards others with simple traces.

Incidentally, it is tempting to follow up the above analogy a little farther. The tissue filling up the peripheral loops of the leaf-trace, and also that enclosed by the pinna- and aphlebia-traces, is in origin a portion of, or at least homologous with, the 'mixed pith' or internal xylem of the stem, while the rest of the trace is its external xylem. Professor Lang (10, p. 239) has already compared the outer and inner xylems of the Ophioglossaceae to the similarly named tissues in the Zygopterideae. The *Clepsydropsis* type of leaf-trace and pinna-trace, which does not cause a gap in the external xylem, may thus stand to the condition seen in *Diplolabis*, &c. (where a gap is caused, for the loop 'opens'), in the same relation as corresponding types of leaf-trace origin observed by Professor Lang in *Botrychium Lunaria* (10, p. 237). The gapless condition would thus by analogy indicate a lower organization than that in which the continuity of the external xylem is disturbed.

In fact, this is another important character upon the basis of which the Zygopterid petioles may be divided into the two groups referred to on p. 374. These two groups may conveniently be called, after their most primitive genera—

1. The Clepsydroideae (including the genera *Clepsydropsis* as now extended, and *Asterochlaena*, Corda), and

2. The Dineuroideae (including *Dineuron*, *Diplolabis*, *Metaclepsydropsis*, ? *Gyropteris*, *Zygopteris*, *Eatapteris*, and *Stauropteris*).

The gap between the two lateral xylem-masses of the Stauropterid leaf-trace must then be compared to the open peripheral loops of the other Dineuroideae.

#### IV. CONCLUSIONS.

1. There are in all Zygopterideae, as in all known plants with pinnate leaves, only *two* rows of pinnae (secondary rachises), one on each side of the leaf. The supposed secondary raches of *Stauropteris*, *Metaclepsydropsis*, *Diplolabis*, *Dineuron*, and *Eatapteris* are really tertiary, and the result of the forking of the true secondary rachises. The latter are completely fused to the primary rachis, but their strands are distinct.

2. This conclusion revives the suggestion (dismissed by Dr. Bertrand as improbable) that *Gyropteris sinuosa*, Goeppert, is a secondary rachis of a form like *Metaclepsydropsis* or *Diplolabis*, in which this organ acquired a cortical sheath independent of the primary rachis.

3. The mode of branching of the *Stauropteris* leaf conforms to a rectangular system (*édification rectangulaire*, P. Bertrand) in which the principal plane of each branch-axis is perpendicular to that of its own mother-axis. The supposed radial symmetry of the leaf-trace in this genus is only superficial.

4. The laminated portions of the Zygopterid leaf were probably held in a more or less horizontal position, with all the segments expanded in the same plane, as in the modern Ferns.

5. Unger's genus *Clepsydropsis* (1856) is extended to include the genus *Ankyropteris*, P. Bertrand (1909), and is divided into two sections named after the two original genera.

The Zygopterideae are divided, on the basis of the vascular structure, into the two sub-families Clepsydroideae and Dineuroideae, after the names of their most primitive genera. In the Clepsydroideae, which are the more primitive group, and include the genera *Clepsydropsis* and *Asterochlaena*, the peripheral loops are permanently closed; the pinna-trace arises as a closed ring, and branches monopodially at the base to supply similarly shaped aphlebia-traces. In the Dineuroideae, including the remaining genera of Zygopterideae, the peripheral loops open at each pinna-trace exit; the pinna-trace is an open arc, and dichotomizes into two equivalent pinnule-traces. These may branch monopodially at the base to supply similar aphlebia-traces. The relations of the genera are shown in the table (p. 376), which is a modification of Dr. Bertrand's latest scheme.

It is my pleasant duty to express my heartfelt thanks to Professor

Seward, who has been a constant source of help and encouragement. This work was carried out during the tenure of a Research Studentship at Emmanuel College, Cambridge, and of a grant from the Dixon Fund of the University of London.

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# The Structure of the Integumentary System of the Barley Grain in Relation to Localized Water Absorption and Semi-permeability.

BY

E. J. COLLINS.

With four Graphs, nine Figures, and one Key Figure in the Text.

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## SECTION I. INTRODUCTION.

THE integumentary system of the cereal grains is a morphological complex resulting from the more or less complete fusion of the various units in the system with each other. In the so-called naked grains the seed-coats and pericarp together form the investment, whilst in the ordinary varieties of barley the flowering glumes are included. As the maturation of the grain proceeds, the system dries and becomes membranous.

Rarely do seeds possess an investment of so complex a nature, and there can be no doubt that it is biologically significant; yet an exploration of the literature dealing with the barley grain showed that no account of the integumentary system was readily accessible, and that the details of its structure and development were very little known.

Holzner and Lermer (1), in their classic account of the barley grain, dealt in some detail with its structure, and the interpretation put upon the structure was adopted very largely by Horace Brown and Morris (2) in their splendid work on the histological and physiological changes which occur at the germination of the grain.

More recently, Adrian Brown's (3) fascinating series of experiments has focused interest on the integumentary system as the seat of semi-permeable properties. The discovery was made when grains of *Hordeum vulgare* var. *caeruleascens* were steeped in sulphuric acid. This particular variety lends itself to an ocular demonstration of the phenomenon, because, if the integumentary system is imperfect, the sulphuric acid enters the grain and the blue pigment contained in the aleurone layers is reddened, whereas grains in which the membrane is perfect swell, but do not show the colour change. More than ordinary interest is attached to this discovery, because it was the first instance of an efficient natural non-living membrane of this type.

From the experimental evidence obtained, Brown assumed that the property resided in the nucellar epidermis, and that water and penetrant substances like iodine entered generally and uniformly all over its surface excepting the flanks of the furrow, where the entry of iodine was delayed.

During the course of the publication of Brown's papers, other workers investigated the phenomenon of semi-permeability in seeds. Amongst these may be mentioned Schroeder (4), Atkins (5), Reichard (6), and Shull (7).

Schroeder, investigating the phenomenon in wheat, showed that a concentration of the solution occurred when grains were immersed in a solution of salt. He endeavoured to correlate the weight increase of the grain with the calculated increase of weight from the increased titre due to the uptake of water by the grain. It will readily be seen that an exact result cannot be expected, for the grain-coverings will increase in weight by the uptake of the salt solution, whilst a number of damaged grains must be allowed for. It is not surprising, therefore, that the actual weight increase was considerably higher than that calculated from the increased concentration of the solution, which of itself indicates penetration of the grain. Further experiments showed that a solution of silver nitrate in 50 per cent. alcohol penetrated the grain, as did osmic acid and iodine in solution, the two latter proceeding from the basal or proximal end, as indicated by the colour change. Watery solutions of chloroform and ether accelerated the uptake

of water, whilst the final weight reached by grains immersed in these solutions was above that of grains steeped in water.

Whilst discussing the various theories advanced to explain the semi-permeability of membranes, Schroeder suggested the possibility of a localized entry, or perhaps an increasing impermeability of the coverings from the basal towards the apical end of the wheat grain. He also suggested the possibility of a reaction between the solution and some constituent of the membrane leading to increasing impermeability, an assumption based upon his experiments with osmic acid.

Reichard, in a first paper, called attention to the presence of a tannin layer in the coverings of the grain of barley, and later, after the discovery of the semi-permeable membrane, sought to connect the presence of tannin with the phenomenon of semi-permeability. As will be seen later, the present writer has failed to confirm the presence of a tannin layer.

Shull dealt particularly with the seeds of *Xanthium*, and concluded that cellulose walls might form efficient semi-permeable membranes. Moreover, in a general review, he regarded semi-permeability in seeds as a widespread phenomenon, and enumerated the various orders and genera of plants whose seeds possessed this type of covering.

Consideration of the investigations that have been made shows that attention has been focused more particularly upon the molecular state of water and the action of the solutes upon this state, a physical rather than a biological study, and that the barley grain has been exploited largely from this point of view. In the present work it is intended to consider the structure and properties of the various layers of the investment, not as a physico-chemical problem, but rather as a biological study in liquid absorption.

The general results of this research may be summarized as follows:

1. Very restricted permeation over the general surface of the grain, either of water or solutes, is indicated.
2. The area of absorption and semi-permeability is located chiefly at the germinal end.<sup>1</sup>
3. The structure of the integumentary system is significant in relation to the path of penetration of water and solutes into the grain.

## SECTION II. STRUCTURE AND REACTIONS OF THE INVESTING INTEGUMENTARY LAYERS.

### (a) *Morphology and structure of the layers.*

For the purpose of determining the structures, grains were steeped in water for two days and sections were cut from the mid region transversely. After a number of trials it was found that sections cut by hand gave the

<sup>1</sup> The germinal, basal, or proximal end of the grain is the end at which the grain is attached to the rachis; the germ is situated on the ventral or curved surface.

most reliable results. The curvature of the grain towards the basal and apical ends made it very difficult to observe the sequence of the layers from sections cut in these regions. First efforts were directed towards obtaining a working knowledge of the structure of the integumentary system, and recourse was had to swelling reagents to facilitate the study of the very much compressed and papery layers. A weak solution of potash in which the sections were left some twelve hours gave the best results, whilst acetic

and lactic acid preparations were a great help. In this way the determination of the precise morphological character of the coverings and their delimitation were made possible as far as the matured grain was concerned.

Fig. 1 is a diagrammatic representation of the layers, after a section of the grain has been swelled by some

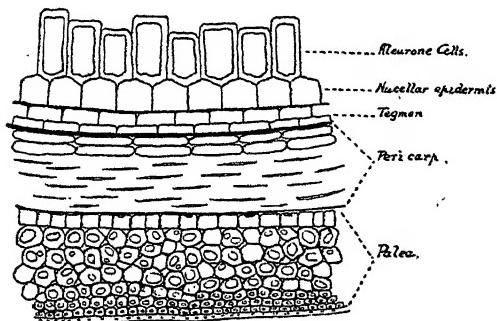


FIG. 1. See text.

twelve hours' immersion in a 3 per cent. solution of potash. The absence of the testa, which becomes disorganized and disappears in the early development of the ovule, must be noticed ; otherwise there is no difficulty

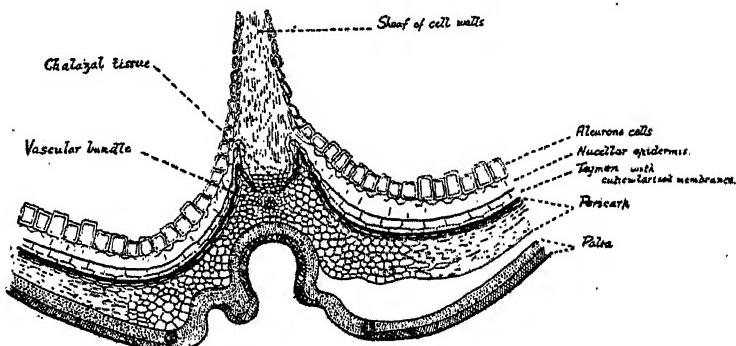


FIG. 2. See text.

in recognizing the sequence of the layers. The nucellar epidermis, invisible in untreated sections, is well marked. The diagram does not indicate the thickness of the walls of the cells of the tegmen.

The structure of the grain in the neighbourhood of the furrow is more complex, as a reference to Fig. 2 will show ; marked changes occur here as maturation proceeds. The furrow corresponds in position and extent with an elongated chalazal tract, through which nutriment and reserve materials

pass from the vascular supply in the ovary wall to the cells of the endosperm. The tissues of the pericarp and ovule are continuous; indeed, this elongated tract is to be regarded as the base of the ovule—the extended chalaza—from the flanks of which the integuments originate. The whole structure recalls very strongly the arrangement found in *Lepidocarpon* and *Lepidostrobus* (8). On the ovarian side of the vascular bundle, and lying immediately between the points of origin of the tegmen, is a group of cells of glandular character with somewhat thickened walls and yellowish homogeneous contents. In the developing grain the contents appear to be of an oily nature. Radiating from this group, towards the centre of the developing grain, is a sheaf-like mass of elongated cells which serve to distribute supplies to the endosperm. In the matured grain these cells are represented by a mass of cell-walls, the cavities of the cells having been obliterated by the compression due to the swelling and subsequent drying of the grain. The sheaf-like mass of cells frequently shows two distributing tracts corresponding to the longitudinal halves of the endosperm on either side of the furrow, and it must further be remarked that the mass extends deepest into the grain where the grain has the greatest circumference (see Figs. 3 and 4).

*The epidermis of the nucellus can be traced immediately within the tegmen up to the point of origin of the latter, but not across the chalazal tract itself.*

It has been usual to regard the whole of the above-described tissues as nucellar in their origin. This interpretation was due to Holzner and Lermer, and is repeated in the work of Brown and Morris and others. A. Brown, in his first communication, suggested that the epidermis of the nucellus functioned as the selective membrane, because the deposit of silver chloride was seen to stop at the base of the sheaf of cells (sheaf-like mass of cell-walls) in the region of the furrow. As the nucellar epidermis does not delimit this sheaf-like mass of cell-walls, it cannot be held responsible for obstructing the passage of the salts at this point.

A structure known as the 'embryonic appendage' must be mentioned here. This is a group of cells forming the apex of the root-sheath, which, lying immediately beneath the micropyle, makes real contact between the embryo and the tegmen. The cells differ from those of the root-sheath proper in that they have no contents and are capable of very rapid swelling when placed in contact with water. Although the actual origin of these cells has not been proved yet, the writer is of the opinion that they may represent a suspensorial group.

(b) *Reaction of the various parts of the integumentary system to reagents.*

Having obtained a knowledge of the various layers and their positions, it was an easy matter to locate any of them in sections which had not been

treated with swelling reagents, but mounted directly in alcohol or glycerine, and the next point determined was their behaviour towards various test reagents.

A consideration of the class of substances able to penetrate the grain led one to think that possibly some lipoid material might be adsorbed by the walls of a particular layer, rendering it semi-permeable and able to function selectively.

Reichard had found tannin to exist in a more or less continuous layer, and later pointed to the possibility of tannin acting when wetted as a colloidal semi-permeable membrane. In order to determine whether tannin was present, the writer soaked whole grains in ferric chloride, but sections cut from them, when examined, showed no reaction attributable to the presence of tannin. Sections were also steeped in ferric chloride, ammonia, copper sulphate, and afterwards examined, but tannin could not be traced.

The results obtained with the more important reagents were as follows :

*Alkanna.* Sections were placed in a freshly prepared solution of alkanna for twelve hours, washed, and mounted in glycerine. The cuticle of the paleae was stained red, as were three other membranes, namely :

- (a) the outermost surface of the pericarp,
- (b) the external surface of the tegmen,
- (c) the internal surface of the tegmen.

The possibility that the layer (c) represents the outer wall of the epidermis of the nucellus has been removed by the examination of the young ovule. The nature of the impregnating material reacting to alkanna was then determined. Fats, wax, suberin, cutin, resin, tannin, and ethereal oils react in this way. By a series of experiments the presence of resin, tannin, or ethereal oils was negatived. Some sections were dried carefully and immersed in chloroform or ether for a lengthy period. On subsequently treating these sections with alkanna or Scharlach R the membranes again reacted, staining red. Hence they were not impregnated with ordinary fatty material soluble in ether. The fat of the aleurone cells had been dissolved.

A further endeavour was made to remove possible fatty material of the membranes enclosing the grain, by placing grains with ether in a Soxhlet tube and allowing extraction to proceed for some weeks. The grains did not lose the wrinkles in the skin and, judging from external appearance only, penetration of the ether into the grain did not occur. The ether was previously deprived of water. Sections taken from the grains and treated with alkanna or Scharlach R gave all the usual reactions, the fat globules of the embryo and aleurone cells, as well as the membranes mentioned above, being stained deep red.

*Alcoholic extract of chlorophyll.* When sections were placed in a freshly prepared solution of chlorophyll in alcohol, the same membranes were stained green. The oil of the aleurone cells was also stained.

*Chlor-zinc-iodine.* Sections mounted in this reagent gave instructive results, as a certain amount of swelling occurred. The three membranes were stained a deep yellow; the walls of which these membranes formed the superficies became blue. In the sub-epidermal layers of the paleae, the blue colouring was pronounced around the cavities of the cells and shaded to the middle lamellae. The perforations in the transverse walls of the large cells of the paleae were made very evident.

*Caustic potash (2·9 per cent. for 12 hours) followed by alkanna.* The course of each of the three membranes was traced in a series of red dots, those of the outer epidermis of the tegmen being much larger than the others; the oil of the aleurone cells was stained red. The nucellar epidermis was made evident.

*Schultze's macerating fluid.* Sections were gradually heated in this fluid on the slide, rinsed in water, and then treated with Scharlach R. The three membranes were stained red, droplets occurring in their course. Droplets also stood at the cut edges of the membranes. This treatment, as also the prolonged treatment with caustic potash, caused the fatty matter impregnating the walls to aggregate.

*Concentrated sulphuric acid.* The membranes resisted this reagent and remained undissolved; the aleurone cells became tinged with pink, probably indicating the presence of a glucoside.

By reason of the tests thus applied the three membranes were judged to be cuticular, the one delimiting the outer border of the tegmen being of considerable thickness.

(c) *Possible alteration of the membrane by penetrating substances.*

An endeavour was made to determine the effect of solutions, more particularly those found to penetrate the grain, upon the cuticular membranes. The method of procedure was as follows: selected sections, not often complete sections of the grain but only of the convex side with the coverings, were placed for varying periods, extending from hours to days, into the solutions. When taken out they were rinsed in distilled water and mounted in glycerine, with or without previous staining with Scharlach R. In this way solutions of ethyl acetate, trichloracetic acid, acetic acid, osmic acid, dilute ammonia, sulphuric, nitric, and hydrochloric acids were tried. No good evidence was obtained that was pertinent to the question of the penetration or non-penetration of these solutions into the grain. The cuticular membranes blackened quickly in osmic acid, while after ten days' immersion in  $H_2SO_4$ ,  $HNO_3$ , and  $HCl$ , of 10 per cent. strength, the membrane gave good reactions with Scharlach R.

## (d) Extent of the cuticularized membranes. (See Figs. 3 and 4.)

The examination of sections cut at different levels between the basal and apical ends of the grain showed that the cuticularized membranes were continuous around the greater part of the grain. The two membranes of the tegmen end in the group of cells in the region of the furrow (see Fig. 2). Sections across the basal end retaining the membranes in position were not easy to secure, so that it was not possible to determine precisely how far the membranes were complete in this region.

Sections of the immature ovule, examined after treatment with Scharlach R., showed that the tegmen membranes were cuticularized even

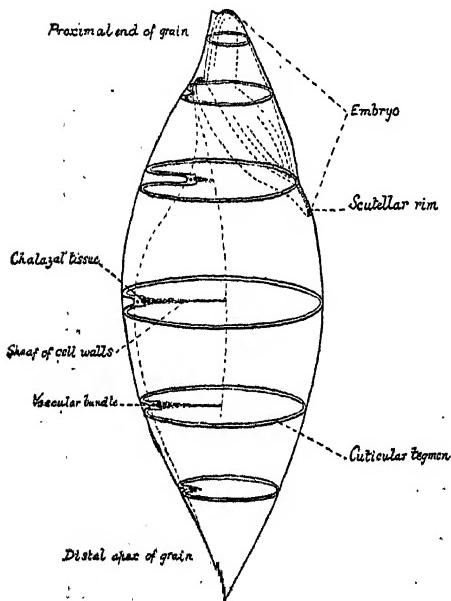


FIG. 3. Diagram of the solid grain with paleae removed.

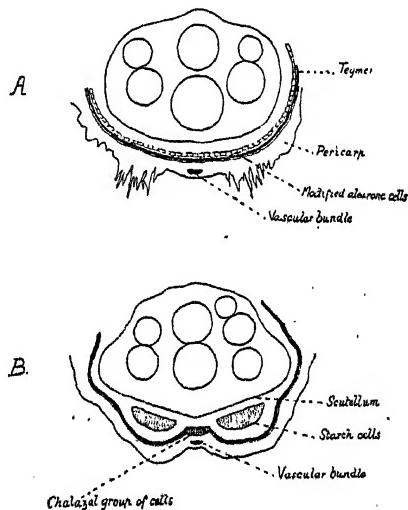


FIG. 4. Diagram of transverse sections of germlinal end of barley grain: A. just above, B. just below, the scutellar rim on the furrow side.

at the earliest stages of development. By means of such sections it was proved that the membranes completely envelop the ovule, with the exception of the micropyle and the chalazal tract itself. In longitudinal sections of the mature grain the micropylar point was not satisfactorily made out, although the membranes could be traced up to a dark-coloured cap-like covering, to which was attached the embryonic appendage.

Protected as it is by the pericarp and paleae, the cuticularization of the tegmen is at once an interesting and suggestive feature in the morphology and biology of the coverings, but for the immediate purpose of this research its physiological significance is in the foreground.

(e) *Impermeability of the cuticular membrane of the tegmen to solutes.*

A. Brown, by using the silver salt method, in which grains were steeped first in a solution of silver nitrate and then in a solution of salt, came to the conclusion that the nucellar epidermis was acting as the semi-permeable membrane. It was a little unfortunate that the region of the furrow was chosen for closest investigation on this point, for, as remarked in a previous section, the nucellar epidermis is not present at this point.

In the experiments recorded here, the same method, with a slightly longer period of immersion in each of the solutions, was followed. The grains were rinsed and exposed to sunlight. Large numbers of sections were cut, always from the middle of the grain, for reasons already stated; the sections were placed directly on slides with water, glycerine, or alcohol, and exposed to strong sunlight until the rims of the sections became quite dark. Some of the best results were obtained from those sections which had stuck to the glass, owing to the evaporation of the alcohol. This, to a great extent, prevented the escape of the particles of silver chloride from the seat of precipitation. The sections were stained with Scharlach R and finally mounted in glycerine. Examination of many sections so prepared left no doubt that the cuticularized outer membrane of the tegmen was responsible for the stoppage of the silver nitrate or the salt, for the deposit of silver chloride could be traced up to this point, but not beyond. In the region of the furrow the yellowish contents of the group of cells situated here were blackened, and in some sections the silver chloride deposit could be seen extending for a little way both into the base of the sheaf-like mass of cell-walls and between the inner and outer cuticularized membranes of the tegmen.

(f) *Summary.*

Investigation of the structure of the integumentary system shows that the barley grain is, with the exception of the micropyle and chalazal tract, completely invested by a strongly cuticularized tegmen membrane. This membrane is destroyed by a solution of caustic potash, which causes the impregnating fatty material to aggregate into droplets; it is not altered when exposed to the action of solutes known to penetrate the grain, and its reactions are not impaired by treatment with solutions of the strong acids. The membrane is impermeable to salt or silver nitrate or both, for after grains have been steeped successively in solutions of these substances, a precipitate of silver chloride can be traced up to the membrane, but not beyond.

**SECTION III. LOCATION OF THE UPTAKE OF WATER BY THE  
BARLEY GRAIN.**(a) *General.*

The possession of such a strongly cuticularized tegmen membrane as has been shown to envelop the grain is very striking. That cuticle is to a certain very small extent permeable to water is established, for cuticular transpiration is a well-recognized phenomenon, and it has been shown that if potassium nitrate is placed on a moistened and wetted area of the cork layer covering the potato, a little water is slowly absorbed from the potato. On the other hand, both cuticle and cork are quite impermeable to many substances. The cuticle of the onion, for example, is impermeable to potassium nitrate, copper sulphate, and mercuric chloride.

A few experiments were made to see how far the cuticle of the upper surface of the leaf of the cherry laurel was permeable. Pieces of the leaf were sealed by wax of low melting-point across the mouth of conical tubes with flanged and ground ends, so that the cuticular surface was exposed to solutions put into the tubes. After exposure to the solution for some days, sections of that part of the leaf exposed to its action were made and examined, but no evidence of penetration was found. In this way resistance to penetration of strong solutions of iodine and trichloracetic acid was determined. Armstrong (9) had previously demonstrated that cuticle was impermeable to solutions of cadmium iodide, acetic acid, and mercuric chloride, amongst other substances, and yet all these substances were found by Brown to penetrate the barley grain.

It is interesting in this connexion to recall the fact of the relatively slow uptake of water by the barley grain, since some six or seven days may elapse before it comes to a maximum weight when steeped in water, whilst split grains reach a similar weight in about thirty-six hours.

From this evidence it was thought possible that there was no marked general permeation of the cuticular investment, and that penetration occurred only at certain spots, and some preliminary experiments were made to test this view. A number of grains was placed in a beaker of distilled water at ordinary laboratory temperature. Every three hours grains were removed from the water, dried carefully, placed between pieces of cork, and cut across the mid region with a dry razor. The cut surface of the grain, which was still held between the pieces of cork, was pressed down upon a piece of dried cobalt paper. The result, after nine hours' immersion, was a print with a pink outline of the grain and a large blue interior. Some measurements were taken of the diameter of the blue centre and the thickness of the pink rim with a millimetre scale, but they are not claimed to be more than rough measurements. Whole grain: 4 mm.  $\times$  3 mm. Blue centre: 3.75 mm.  $\times$  2.5 mm. Tests were made between nine and twenty-four hours, and grains were obtained, after twenty

hours' immersion in water, whose cross-sections treated in the manner described gave a print with a pink rim and a large blue interior. If sections were taken nearer the germinal end, however, the whole print of the cross-section was pink, showing that in this region the tissue had become wetted. The evidence thus gained pointed to localized entry at the basal end of the grain and at once suggested the micropyle, since at this point the cuticularized tegmen was judged to be incomplete.

An endeavour was made to block the micropyle in some way by means of waterproofing solutions, and, by a comparison of the weight-increase of such grains when placed in water with that of normal grains, to confirm this point. Individual grains were taken and the basal tip of each grain—paleae and embryonic tip—was cut off. Each grain was now weighed, and the cut tip was painted with one or more of the various solutions, e.g. sealing-wax dissolved in alcohol, fortifix, and rubber solution, allowed to dry, and weighed again. After this they were placed apex down in small holes in cork rafts floated on water, and with the control grains maintained at 20° C. Many trials were made, but the result of one set only is given here.

10 grains, cut as described and sealed with a solution of sealing-wax overlaid with rubber solution.

No.	Weight with Seal.	Weight After 8 hrs.	After 24 hrs.	After 32 hrs.	Increase 8 hrs. absolute and %	Increase 24 hrs. absolute and %	Increase 32 hrs. absolute and %
1	0.0465	0.0495	0.0550	0.0605	55 = 11.8	110 = 23.6	135 = 29.0
2	0.0550	0.0580	0.0630	0.0705	50 = 9.0	125 = 22.7	145 = 26.0
3	0.0505	0.0540	0.0595	0.0660	55 = 10.8	120 = 23.7	145 = 28.7
4	0.0525	0.0550	0.0600	0.0660	50 = 9.5	110 = 20.9	140 = 26.6
5	0.0495	0.0520	0.0575	0.0625	55 = 11.2	105 = 21.2	135 = 27.2
6	0.0545	0.0565	0.0620	0.0685	55 = 10.0	120 = 22.0	150 = 27.5
7	0.0560	0.0580	0.0635	0.0700	55 = 9.8	120 = 21.4	155 = 27.6
8	0.0500	0.0520	0.0560	0.0615	40 = 8.0	95 = 19.0	125 = 25.0
9	0.0515	0.0530	0.0585	0.0660	55 = 10.6	130 = 25.2	155 = 30.0
10	0.0545	0.0560	0.0650	0.0750	90 = 16.5	190 = 34.8	215 = 39.4
Omitting No. 10, average increase					10 %	22.1 %	27.5 %

5 control grains; not cut and not sealed.

1	0.0510	0.0610	0.0690	0.0710	100 = 19.6	180 = 35.3	200 = 39.2
2	0.0475	0.0560	0.0605	0.0635	85 = 17.8	130 = 27.3	160 = 33.7
3	0.0510	0.0610	0.0660	0.0690	100 = 19.6	150 = 29.4	180 = 35.3
4	0.0445	0.0525	0.0585	0.0605	80 = 17.9	140 = 31.4	160 = 35.9
5	0.0465	0.0565	0.0635	0.0660	100 = 21.5	170 = 36.5	195 = 41.9
Average increase					19.3 %	32.0 %	37.2 %

5 control grains; cut only.

1	0.0565	0.0795			230 = 40.7	
2	0.0535	0.0735			200 = 37.3	
3	0.0490	0.0715			225 = 46.0	
4	0.0480	0.0700			220 = 45.8	
5	0.0460	0.0695			235 = 51.0	

The average increase for 24 hours is 44.2 per cent.

From these results it will be seen that the sealing is effective in reducing the quantity of water absorbed.

Controls treated in precisely the same manner were set up in iodine. Blueing of the grain in the germinal region showed that the sealing was not effective in preventing the ingress of liquid. It was found that the swelling of the coats of the grain broke the sealing, and thus paved the way for the entry of liquid. However, a difference in the rate of uptake was clearly obvious as a general result of the long series of experiments; moreover, the possibility of the entry of liquid at special points in the germinal region, other than the micropyle, must not be overlooked. In this connexion it must be mentioned that at the germ end of the furrow, where the scutellum comes practically to the surface of the grain, there is no mass of cell-walls to hinder the passage of liquids once they have traversed the group of cells with yellowish contents. Frequently, evidence was obtained that penetration occurred at this point (see Figs. 3 and 4 B).

(b) *Uptake by germinal and apical portions of the grain compared.*

To secure, if possible, some further confirmation of the view put forward that the uptake of water is localized somewhere in the germinal region of the grain, recourse was had to the following indirect method: Individual grains were selected and weighed; as soon as weighed, each was placed in distilled water contained in a numbered watch-glass with a cover. All were maintained at a temperature of 22° C. At the end of each hour a grain was taken, dried externally, and weighed. It was divided into two portions by a transverse cut just above the embryo. Each portion was weighed and then allowed to dry in the laboratory at the ordinary temperature, about 22° C. After drying, the portions were weighed again. In this way the increase of weight per hour of each portion of the grain was obtained, and a comparison instituted.

The value of the uptake of water by the coverings was estimated by Brown at 8 per cent. of the dry weight, and this must be borne in mind when considering the results. The following tables give the results of a series of experiments, and a set of curves has been plotted to show the results of each series graphically. A second base line, representing the value of the uptake of water by the coverings, has been drawn in the graph series.

SERIES I. GRAINS FROM ONE EAR.

No.	Dry Weight.	Hrs. Steep.	Wet Weight.	Increase.	%	Germ Portion. Dry.	Germ Portion. Wet.	%	Apical Portion. Dry.	Apical Portion. Wet.	%
I	0.0670	I	0.0715	0.0045	6.7	0.0250	0.0275	10.0	0.0410	0.0420	2.4
2	0.0665	2	0.0720	0.0050	9.0	0.0155	0.0185	19.3	0.0500	0.0510	2.0
3	0.0650	3	0.0710	0.0060	9.0	0.00975	0.0125	22.0	0.05425	0.0570	5.0
4	0.0660	4	0.0765	0.0075	10.8	0.0120	0.0160	37.5	0.0560	0.0590	5.3
5	0.0660	6	0.0755	0.0095	14.4	0.0160	0.0220	37.5	0.0490	0.0520	5.7
6	0.0640	7	0.0730	0.0090	14.0	0.0175	0.0230	31.4	0.0455	0.0485	6.5
7	0.0635	8	0.0735	0.0100	15.7	0.0155	0.0220	41.9	0.0475	0.0500	5.2
8	0.0665	9	0.0765	0.0100	15.0	0.0195	0.0255	30.7	0.0460	0.0490	6.5
9	0.0680	11	0.0800	0.0120	17.6	0.0240	0.0320	33.3	0.0435	0.0470	8.0
10	0.0610	12	0.0715	0.0105	17.2	0.0180	0.0250	38.8	0.0420	0.0455	8.3
11	0.0640	24	0.0825	0.0185	28.9	0.0225	0.0325	44.4	0.0405	0.0490	20.9
12	0.0675	25	0.0865	0.0190	28.1	0.0215	0.0320	48.8	0.0455	0.0530	16.4

SERIES II. GRAINS FROM DIFFERENT EARS.

No.	Dry Weight.	Hrs. Steep.	Wet Weight.	Increase.	%	Germ Portion. Dry.	Germ Portion. Wet.	%	Apical Portion. Dry.	Apical Portion. Wet.	%
1	0.0525	1	0.0565	0.0040	7.6	0.0105	0.0130	23.8	0.0410	0.0430	4.8
2	0.0620	2	0.0690	0.0070	11.2	0.0105	0.0140	33.3	0.0505	0.0545	7.9
3	0.0570	3	0.0530	0.0060	12.7	0.0110	0.0145	31.8	0.0350	0.0380	8.5
4	0.0680	4	0.0775	0.0095	13.9	0.0170	0.0215	26.4	0.0505	0.0550	8.9
5	0.0515	6	0.0615	0.0100	19.4	0.0165	0.0220	33.3	0.0350	0.0385	10.0
6	0.0660	7	0.0770	0.0110	16.6	0.0155	0.0215	38.7	0.0495	0.0545	10.1
7	0.0505	8	0.0605	0.0100	19.7	0.01575	0.0220	39.0	0.03375	0.0380	12.5
8	0.0585	9	0.0695	0.0110	18.8	0.0195	0.0265	35.8	0.0380	0.0425	11.8
9	0.0570	11	0.0690	0.0120	21.0	0.0205	0.0270	32.1	0.0360	0.0415	15.2
10	0.0500	12	0.0620	0.0120	24.0	0.0225	0.0300	33.3	0.0270	0.0310	14.8
11	0.0450	24	0.0600	0.0150	33.3	0.0160	0.0255	59.3	0.0280	0.0340	21.4
12	0.0565	25	0.0725	0.0160	28.3	0.0255	0.0360	41.1	0.0300	0.0360	20.0

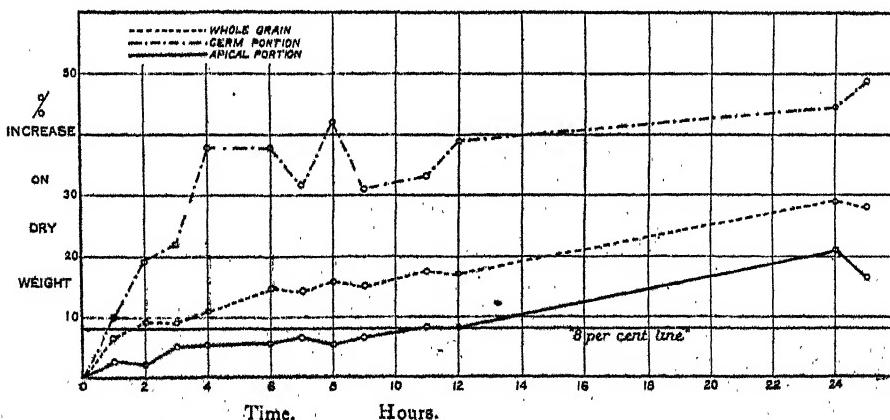
SERIES III. GRAINS FROM ONE EAR.

1	0.0560	1	0.0600	0.0040	7.1	0.0115	0.0130	13.0	0.0440	0.0465	5.6
2	0.0540	2	0.0600	0.0060	11.1	0.0105	0.0130	23.8	0.0440	0.0470	6.8
3	0.0530	3	0.0595	0.0065	13.2	0.0085	0.0110	29.4	0.0440	0.0480	9.0
4	0.0515	4	0.0590	0.0075	14.5	0.0145	0.0190	31.0	0.0365	0.0395	8.2
5	0.0520	6	0.0620	0.0100	19.2	0.0165	0.0220	33.3	0.0340	0.0390	14.7
6	0.0530	7	0.0635	0.0105	19.8	0.0130	0.0190	46.1	0.0395	0.0440	11.3
7	0.0510	8	0.0615	0.0105	20.5	0.0130	0.0195	50.0	0.0370	0.0410	10.8
8	0.0490	9	0.0600	0.0110	22.4	0.0130	0.0195	50.0	0.0355	0.0395	11.2
9	0.0500	11	0.0610	0.0110	22.0	0.0130	0.0195	50.0	0.0365	0.0405	10.9
10	0.0490	12	0.0610	0.0120	24.4	0.0125	0.0195	56.0	0.0355	0.0405	14.0
11	0.0470	25	0.0645	0.0175	37.3	0.0155	0.0250	61.0	0.0305	0.0355	26.0

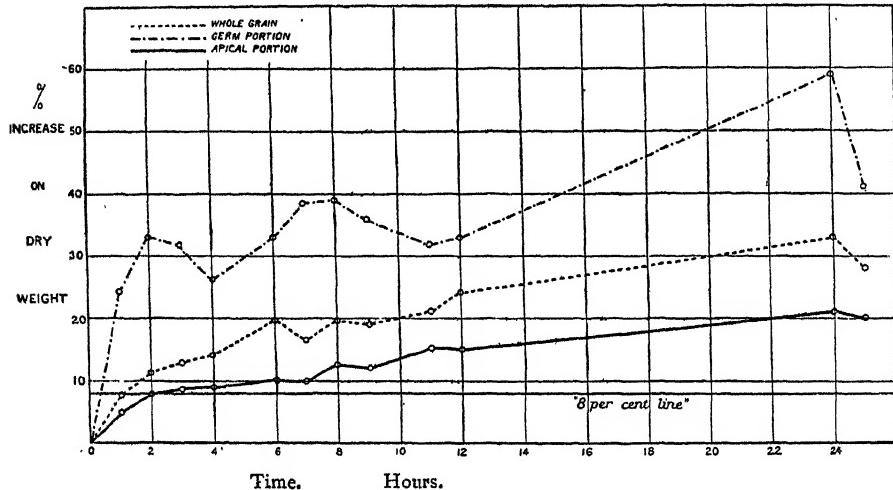
SERIES IV. GRAINS FROM ONE EAR. 12-24 HOURS.

I	0.0595	12	0.0745	0.0150	25.2	0.0210	0.0290	38.0	0.0380	0.0450	18.4
2	0.0610	13	0.0750	0.0140	22.9	0.0215	0.0290	34.8	0.0385	0.0450	16.8
3	0.0615	14	0.0775	0.0160	26.0	0.0250	0.0345	38.0	0.0355	0.0420	18.3
4	0.0610	15	0.0780	0.0170	27.8	0.0235	0.0330	40.4	0.0305	0.04475	22.6
5	0.0605	16	0.0750	0.0145	23.9	0.0225	0.0315	40.0	0.0370	0.0430	16.2
6	0.0580	17	0.0755	0.0175	30.1	0.0190	0.0290	52.6	0.0380	0.0460	21.0
7	0.0590	19	0.0760	0.0170	28.8	0.0240	0.0350	45.8	0.0340	0.0405	19.1
8	0.0595	20	0.0780	0.0185	31.0	0.0225	0.0325	44.4	0.0360	0.0450	27.7
9	0.0560	21	0.0735	0.0175	31.2	0.0205	0.0305	48.7	0.0345	0.0420	21.7
10	0.0560	23	0.0760	0.0200	35.7	0.0185	0.0290	56.7	0.0365	0.0460	26.0
11	0.0530	24	0.0860	0.0230	36.5	0.0200	0.0310	55.0	0.0415	0.0540	30.1

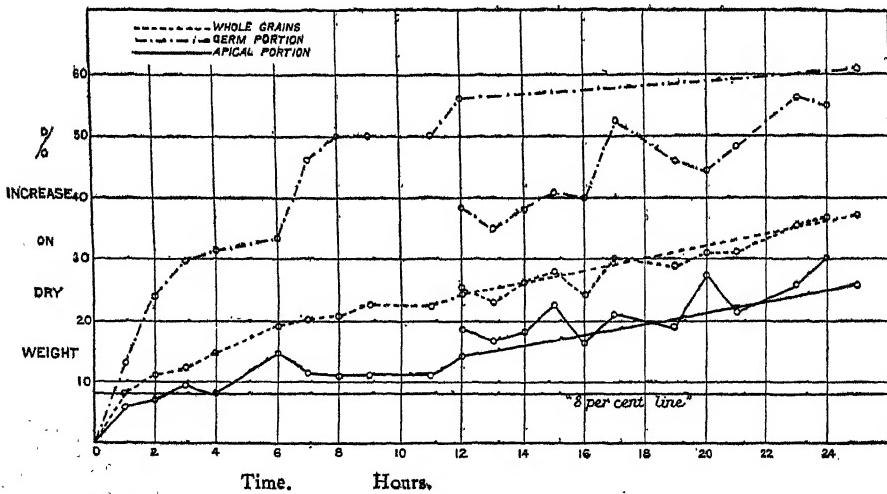
Barley grains: Uptake of water related to increase of weight by the two halves of the grain. Series I.



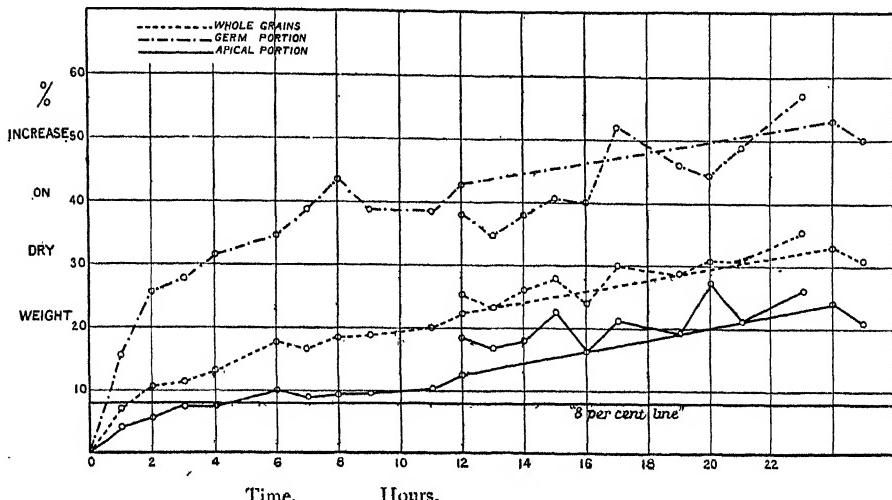
Barley grains: Uptake of water related to increase of weight by the two halves of the grain. Series II.



Barley grains: Uptake of water related to increase of weight by the two halves of the grain. Series III and IV.



Barley grains: Uptake of water related to increase of weight by the two halves of the grain. Average of Series I, II, III, and IV.



From a consideration of the tabulated results, it will be seen that the original weights of individual grains varied within and between the series; the weights of the grains in Series I were more uniform. Naturally, the lack of uniformity in weight, size, and integuments will tend to obscure the results somewhat, particularly when percentages of dry weight are made the basis of comparison. The impossibility of cutting the grains so that each portion in every grain bears the same relative proportion to the whole adds still further to the difficulty of comparison. Although the grains themselves may vary largely in size and weight, the embryo is most likely to be of uniform proportion, for during its development, even in the smallest grain, there will be an abundance of food material, and consequently the embryo may be expected to fluctuate but little in size. Having due regard to these facts, it is claimed that the absolute increases in weight of the grains, irrespective of the original weights, afford a comparable series, and from this the conclusion is drawn that the initial uptake of water supplies in the main the needs of the embryo itself.

These results can only be interpreted in the sense that the germinal portion of the grain increases in weight much more rapidly than the apical portion, despite the fact that, in the way the grains were divided, the apical portion would possess by far the greater surface area. The water must come to the embryo either by local absorption at one or more points, or, after having been absorbed generally over the whole surface, be passed directly to the embryo without passing to and wetting the starchy endosperm. There was no evidence for the latter view, and when the distribution

of liquids within the grain is considered it will be shown that distribution is always from the germinal towards the apical end.

(c) *Degree of permeability of the cuticularized integument to water.*

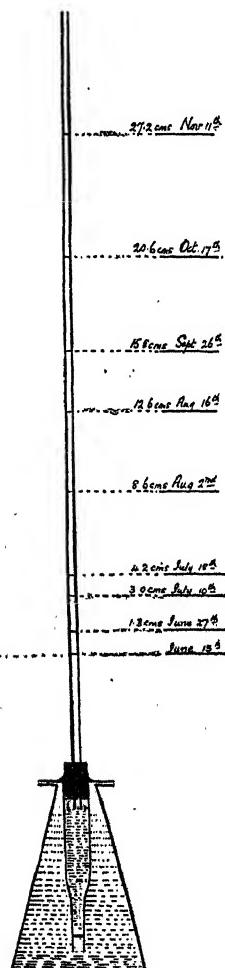
Many endeavours were made to make use of a portion of the coverings of the grain as a septum in an osmotic cell, in order to study the degree to which the cuticularized membranes were permeable to water. One attempt proved successful.

For this purpose grains were soaked for a short time in water, dried superficially, and halved longitudinally in a plane at right angles to the furrow. The half with the convex external surface was then flattened somewhat by gentle pressure and a circular portion cut out by means of a sharp cork-borer. This thick circular plate was painted very carefully round the edge with rubber solution and pushed into a slightly tapering tube by a glass rod. By this means the circular piece of grain-covering, with some amount of the endosperm attached, was caused to make good contact with the walls of the glass tube. To obtain an osmotic suck, the tube was filled with a 20 per cent. solution of salt. The lower end was immersed in distilled water in an open beaker, whilst the upper flanged end was prolonged by a tube of narrow bore carried in a rubber cork. (See Fig. 5.) Air within the tubular portion below the membrane was removed. Starch and aleurone cells were on the upper side of the membrane exposed to the salt solution.

The apparatus was set up on June 13, 1914. Five days elapsed before any rise was noticed in the level of the salt solution within the tube. After this the rising continued very slowly. On the eleventh day the

FIG. 5. Diagram of osmotic cell.

external water was tested for salt, but none was found. After thirty-five days the level had risen 4·2 cm. in a tube of 2·5 mm. bore, and, as traces of salt were found in the distilled water outside, the tube was placed in a saturated solution of chloroform water contained in a conical flask whose



TABULAR STATEMENT OF THE DATA OBTAINED AND CALCULATIONS MADE THEREFROM.

Duration of experi- ment in days.	Interval between readings in days.	Rise of level in mm.	Volume equivalent to rise, in c.c.m.		Volume passed, in c.m.m. per sq. mm. of membrane.	Whole period.	Whole interval.	Volume passed per day, in c.m.m. per sq. mm. of membrane.
			Whole period.	Interval.				
o <sub>10</sub> 14	...	o	...	...	...	...	...	...
	14	13	13	63.7	4.55	6.6	o.47	o.66
27	...	17	...	83.3	6.4	8.6	...	...
	30	...	147	...	5.4	15.3	o.56	...
35	8	...	12	58.8	...	7.6	6.1	o.76
	42	...	205.8	...	5.8	21.4	o.61	...
50	15	...	44	215.6	...	14.3	22.4	o.49
	86	...	421.4	...	8.4	43.9	o.87	...
64	14	...	40	196	...	14.0	20.4	1.45
	126	...	617.4	...	9.6	64.3	1.0	...
During the writer's absence from the laboratory and before the following reading was taken, a bubble of air or gas had collected immediately below the septum. The bubble was removed after the reading had been recorded.								
105	41	32	156.8	...	3.8	16.3	o.4	...
	...	75.8	74	...	7.3	80.6	o.76	...
126	21	48	235.2	...	11.2	24.5	1.16	...
	...	266	1009.4	...	8.0	105.1	o.83	...
151	25	66	323.4	...	12.9	33.6	1.34	...
	...	272	1332.8	...	8.8	138.7	o.91	...

mouth was covered by the flange of the tube. After four months the height attained in the osmometer tube was 20·6 cm. and traces of salt were found in the chloroform water. Iodine was now added to the chloroform water and in a few days a layer of starch attached to the membrane was coloured blue. Five months from the commencement of the experiment the height reached in the tube was 27·2 cm.; about one-half of the whole starch layer had been coloured by the iodine.

At the time the experiment was planned it was thought desirable to see whether an isolated portion of the grain-covering could be used to demonstrate semi-permeability. Moreover, if the result was satisfactory, a useful comparison between the rate of passage of water across the barley septum used in the osmometer tube and the rate of the uptake of water by barley when steeped could be made. From this point of view the results were not satisfactory, for the calculations made revealed the fact that the rate at which water passed across the septum was continually accelerated notwithstanding the accompanying dilution of the salt solution, and it was evident that, owing to some alteration, the membrane became more and more permeable as the experiment progressed. It must be recalled that, although the grain had been steeped 12 hours before the septum was cut from it and fixed, no rise in the level of the salt solution was evident for the first five days of the experiment, a fact in harmony with the theory advanced in this paper, that the cuticularization of the tegmen prevents the immediate absorption of water over the general surface of the grain.

(d) *Efficiency of the superficial structures of the grain in capillary conduction of liquids.*

If the apex of the grain dips into water or other liquid, the liquid quickly travels to the germinal end. The outer paleae are very efficient capillary conductors, and act so because of the porous character of the thin-walled tissue beneath the fibrous sub-epidermal cells, the openings in the epidermis being accessory to this function. When the apex of the grain is touched with a drop of coloured alcoholic solution, the colouring is seen to spread towards the germ end with surprising rapidity. The paths principally taken are the furrow and the overlapping edges of the inferior palea. The furrow, wide at its apical end, narrows and deepens quickly towards the germinal end, where it is roofed by the hairy rachilla, and is thus built on a plan suitable for the rapid conveyance of liquid. The whole integumentary system seems admirably adapted for the absorption and conduction of water to the point where it most readily finds admittance to the seed.

To obtain some idea of the efficiency of this conducting mechanism, the relative uptake of water by (a) grains wholly immersed, (b) grains whose germinal end just dipped into water, (c) grains whose apex just

dipped into water, was determined. Five grains were taken in every case, and the temperature was maintained at 20° C.

In one experiment of 15 hours' duration, the grains, whether the germinal or apical end was immersed, increased 22 per cent. in weight, whilst the wholly immersed grains gained 24.5 per cent. in weight.

The results afforded a striking confirmation of the efficiency of the coverings in the capillary conduction of liquids, allowance being made for the fact that the wholly immersed grains would probably absorb more in the coverings. Moreover, this efficiency explained the difficulty experienced in carrying out the critical location experiments which have been described.

#### IMMERSED WHOLLY.

##### SERIES I.

No.	Weight.	Hours' steep	Increase.	%	Weight.	Hours' steep.	Increase.	%
1	0.0565	20½	0.0195	34.5	0.0635	15	0.0155	24.4
2	0.0555	"	0.0185	33.2	0.0560	"	0.0130	23.2
3	0.0590	"	0.0190	32.2	0.0610	"	0.0155	25.4
4	0.0610	"	0.0190	30.8	0.0580	"	0.0155	26.7
5	0.0500	"	0.0155	31.0	0.0585	"	0.0135	23.0

Total grain weight, 0.2885.  
Average % increase, 32.3%.

Total grain weight, 0.2970.  
Average % increase, 24.5%.

##### GERM END IMMERSED.

1	0.0640	20½	0.0190	29.6	0.0625	15	0.0135	21.6
2	0.0645	"	0.0185	28.6	0.0575	"	0.0125	21.7
3	0.0620	"	0.0175	28.2	0.0615	"	0.0125	24.3
4	0.0670	"	0.0195	29.1	0.0580	"	0.0125	21.5
5	0.0650	"	0.0181	27.7	0.0590	"	0.0125	21.1

Total grain weight, 0.3225.  
Average % increase, 28.6%.

Total grain weight, 0.2985.  
Average % increase, 22.0%.

##### APEX IMMERSED.

1	0.0550	20½	0.0150	27.2	0.0630	15	0.0130	20.6
2	0.0625	"	0.0160	25.6	0.0550	"	0.0125	22.7
3	0.0510	"	0.0155	30.3	0.0610	"	0.0155	25.4
4	0.0590	"	0.0155	26.2	0.0570	"	0.0115	20.1
5	0.0605	"	0.0165	27.2	0.0610	"	0.0130	21.3

Total grain weight, 0.2880.  
Average % increase, 27.3%.

Total grain weight, 0.2970.  
Average % increase, 22.0%.

#### SECTION IV. LOCATION OF THE UPTAKE OF SOLUTES BY THE GRAIN.

(a) *Uptake of iodine and its distribution in the grain.* (See Figs. 6, 7, 8.)

Schroeder, dealing with the penetration of iodine in wheat grains, stated that the blueing of the starch contents of the grain was always first noticeable on the shoulders of the germinal end at each side of the furrow, and from this point spread gradually. Brown, dealing with the same point,

namely, the penetration of iodine into the barley grain, stated that 'the blueing of the starch grains renders it easy to study the manner in which iodine passes into the grain, and indicates that it penetrates all parts of the skin enveloping the endosperm at approximately the same velocity, with the exception of the part in the neighbourhood of the furrow, through which it appears to pass with difficulty'.

The results obtained by the writer with barley are quite opposed to Brown's.

Because of the results obtained in the experiments dealing with the uptake of water, it was thought advisable to repeat the barley and iodine experiments; grains of both *Hordeum vulgare* var. *caerulescens* and *H. vulgare* var. 'Goldthorpe' were placed in solutions of iodine in potassium iodide. In both cases the first signs of blueing of the starch contents

appeared on each side of the furrow at the germ or basal end. (See Fig. 6.) From these points it was easy to trace the path taken by the iodine. Whilst it spread to some extent along each side of the furrow towards the apex of the grain, it passed more rapidly around the starchy endospermic rim, immediately beneath the scutellum. The two blue arcs joined round the embryo, completing the circuit; at the same time the blueing spread rapidly on the curved side of the

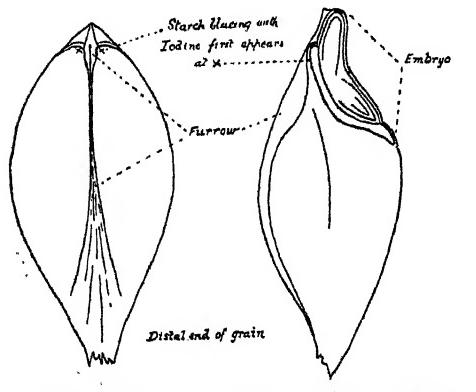


FIG. 6. Grain with paleae removed.

grain towards the apex, the lateral edge of colour as a rule being somewhat sharply delimited. The flanks of the furrow side were the last to be blued. The stronger the solution of iodine, the more rapidly the blueing occurred, an immersion of 12–24 hours in a very strong solution of iodine sufficing for complete coloration, but the path travelled was always the same except when penetration occurred through the presence of injuries.

The first trials were made with grains wholly immersed in the solution, but in order to confirm the results many further experiments were made and are described below.

Grains were placed distal apex downward in holes in cork rafts about two inches square. The rafts were then floated on iodine solution contained in covered Petri dishes. The iodine was thus brought into contact with the apex of the grain, perhaps a quarter of the grain being actually in contact with the solution. Every opportunity was thus given for penetration of the solution into those parts with which it came into contact first. Never-

theless in the course of a few hours two blue points, situated on either side of the furrow at its germinal end, indicated the penetration of the iodine to the starchy endosperm. Spreading quickly from these points the coloration proceeded distally as above described, stopping on the furrow side after a band of a certain breadth had been coloured. Fig. 7 represents the distribution of the colour change in a series of barley grains after

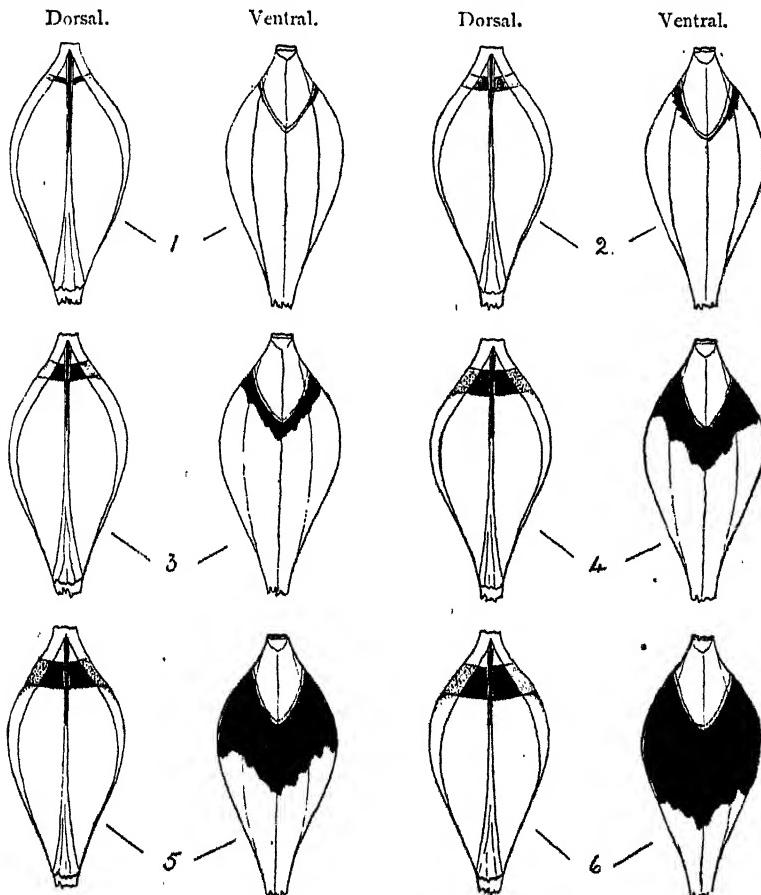


FIG. 7. Distribution of iodine. Exterior view.

increasing periods of immersion in a solution of iodine. Both curved and furrowed side of each grain are shown.

There is but little doubt that non-coloration of the starchy endosperm on the flanks of the furrow is due either to the peculiar configuration of the tissues in this region, which prevents the rapid penetration of liquids in this direction, or the absence of the small sub-aleuronic cells with small starch grains along which the iodine quickly makes its way, or both. Experiments show that these cells imbibe and swell rapidly, and it is

usually held that of the starchy cells of the endosperm, this layer at least remains living.

Fresh unripe, but well-developed grains were also experimented with; in all cases the phenomena observed were similar to those described for the dry mature grain when put into contact with iodine.

Wheat and oats exhibited the same general phenomena.

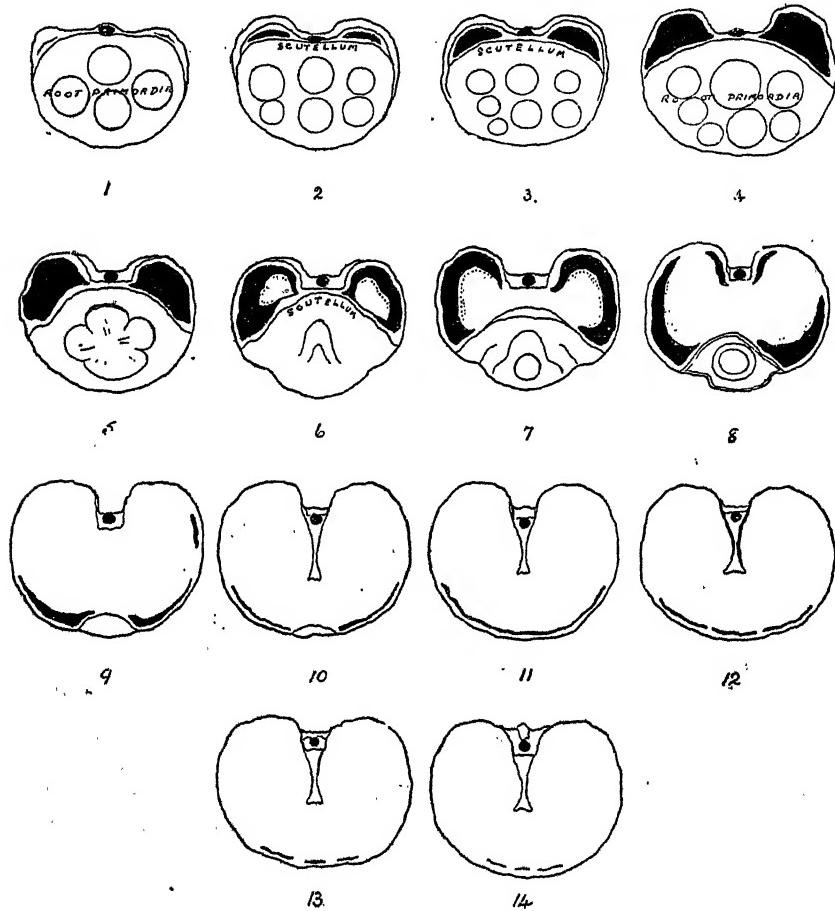


FIG. 8. Distribution of iodine in endosperm. Serial transverse sections from a grain steeped in iodine solution. The blackened areas, except those representing the chalazal tissues, indicate the coloration of starch by iodine. (See Key Fig. 8a.)

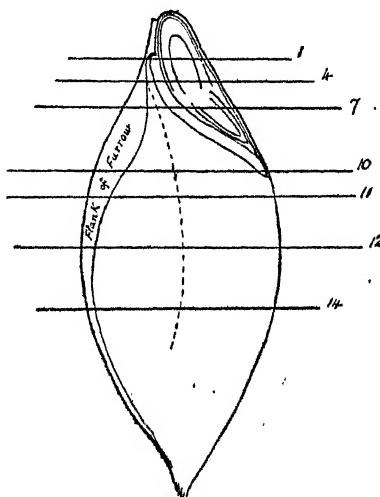
A further test was made by embedding grains in iodine-gelatine. A strong solution of iodine was made up with 12 per cent. gelatine, the iodine solution being added just before the gelatine set. The grains were immersed and arranged in rows in the glass dish. The diffusion of iodine was slower and the process of blueing was easily watched; it proceeded in the way already described and figured. Another advantage lay in the

method, for it enabled one to test the grain with regard to imperfections in the membrane, for wherever a fault occurred a black patch appeared. Since such flaws in the grain-coverings affect the results in all estimations of the semi-permeability of the barley by steeping methods, a test of this kind should be made with a sample of the grain to be used. A few tests made by the writer showed that appreciable differences existed in the various samples used.

With the hope of tracing the path taken by the iodine, both transverse and longitudinal sections of the grain after immersion in the iodine were made. Leaving for a moment the very difficult question of its mode and place of entry and circulation in the embryo itself, it was quite clear that after the initial coloration of the starch in the neighbourhood of the germinal end of the furrow and around the scutellar rim, the layer of small cells with small starch granules immediately subjacent to the aleurone layers was the first to show the effects of the iodine. Indeed it can be said that the iodine travelled along this layer before spreading to any extent towards the centre of the grain. The stronger the solution of iodine used the more quickly did the blueing spread, and the absence of this layer from the region of the flanks of the furrow seems a sufficient reason for the tardy coloration of this area. During the coloration of this layer, blueing spread across the surface of the endosperm cells below and parallel to the surface of the scutellum. There was good reason to suppose that the iodine did not find a ready path along the aleurone cells, for, although they became yellowed progressively in the distal direction, the blueing of the adjacent starch grains proceeded more rapidly.

Fig. 8 shows a number of transverse sections cut from the grain after steeping in iodine solution; the blackened areas indicate the distribution of colour in the starchy endosperm. The approximate position of these sections is indicated in Key Fig. 8a.

From the fact that iodine is adsorbed by the starch grains, wetting by water probably precedes coloration by iodine. If this be so, then the rapidity with which water normally passes along the peripheral layer of the starchy endosperm is striking.



KEY FIG. 8a. Diagram sketch of a median longitudinal section of the barley grain, upon which are plotted the approximate positions of the sections shown in Fig. 8.

In the embryo, the furrow side became coloured yellow. It is presumed that iodine passed to it from a point at the germinal end of the furrow. The yellow coloration spread round the rim of the scutellum; the radicle with the root-cap and sheath became browned very early, probably by reason of the entry of iodine from the micropyle, while the plumule with its sheath, although apparently turgid, and the scutellum, with the exception of the rim, were the last to be coloured. Whilst it is presumed that the entry of iodine took place at two points, it is equally evident that the progress of coloration can also be explained on the assumption of its entry at the micropyle alone with subsequent peripheral distribution in the distal direction.

The result of this series of experiments is to show that the entry of iodine solution into the barley grain is not general and uniform over the whole surface of the grain, but that its uptake is localized at the germinal end, and that it is distributed peripherally in the endosperm along the subaleuronic layer of starch cells of the curved surface.

(b) *Uptake and distribution of some acids.*

When grains were steeped in aqueous solutions of acids it was found that the rate at which the different acids were able to penetrate the grain varied considerably. The entry of sulphuric acid was so slow that a marked concentration of the solution took place; with acetic acid and nitric acid a *weakening* of the strength of the solution occurred, owing to the more rapid penetration of the acid. The concentration effect with sulphuric acid was only temporary, and passed away as the grain reached its maximum increase in weight.

The following experiment, with barley of the current year, is representative of a number made with grain immersed in a solution of sulphuric acid. 150 grm. of grain were steeped in 250 c.c. of 4·9 per cent.  $H_2SO_4$ , and maintained at 20° C. approximately. 1 c.c. of the acid neutralized 7·5 c.c. of a solution of KOH. The titre of acid in contact with barley is given in the third line. At the same time the percentage weight increase of barley soaked in a sulphuric acid solution of similar strength was taken.

No. of Exper.	Time.	<i>Hrs.→</i>	<i>Days→</i>											
			0	12	24	36	48	72	96	9	16	28	35	49
6	Acid Control		7·5	7·48	7·5	—	—	7·52	—	—	—	—	—	—
6	Acid with Barley	,		7·82	8·26	8·36	8·4	8·4	8·34	8·04	7·6	7·3		
	Increase of titre	+		0·34	0·76	0·86	0·9	0·88	0·84	0·54	0·1	=0·2		
	% increase weight of barley steeped in $H_2SO_4$	0		31·5		38·0				59·0	62·0	65·0		
	% increase when steeped in water						61·0			65·5				

Similar experiments with solutions of acetic and nitric acids showed an initial weakening effect, but the subsequent action was obscure; further experiments will be necessary to elucidate this.

It will be seen from these results that when grain is immersed in a solution of sulphuric acid, water initially penetrates faster, whilst the acid penetrates relatively faster as the period of steeping is prolonged, hence the 'concentration effect' at any time measures the temporary balance and cannot have any static significance into which a time factor does not enter.

In tracing the path of entry into and the distribution of acids in the grain, the blue variety of barley was particularly useful. Acetic, trichloracetic, lactic, sulphuric, and nitric acids were experimented with. With the first two acids, solutions of which Brown found to penetrate the grain rapidly, a colour change was first noticeable at the germ end of the furrow; from this point the pink coloration due to the acid reaction with the blue pigment contained in the aleurone cells proceeded round the rim of the endosperm, spreading slowly in the distal direction at the same time. Progress of the coloration was more rapid on the curved side. In general the mode of entry and path of distribution of these acids were identical with those of iodine. With lactic acid, owing to its slower penetration, the colour reaction was not so well marked, but there was sufficient evidence to show that entry was obtained and distribution occurred as indicated for acetic and trichloracetic acids. In grains immersed in sulphuric acid solution the "colour change could not be followed with certainty, as selected grains had to lie for a long time before any distinct signs of entry were manifest. Changes occurred meanwhile which obscured the acid reaction. It was thought that in some grains entry occurred through passage of the tissues of the furrow.

With regard to nitric acid, Brown stated that entry was gained at a point at the germ or proximal end, and that penetration was due to the destruction of the membrane.

So far as this statement relates to the mode of entry of a penetrant solute, it supports the theory put forward by the present writer, whose experiments further showed that immersion in nitric acid neither destroyed the enveloping membrane nor impaired the efficiency of the selective tract, whatever this might be. The evidence upon which this assertion is based will now be detailed.

Grains, after immersion in 1, 5, or 10 per cent. solutions of nitric acid for considerable periods, with subsequent soaking in water and drying, were placed in iodine solution. In all cases the first appearance of starch blueing occurred in the usual place, namely, the germinal end of the furrow and coloration proceeded in the usual way. It was evident that the general impermeability of the membrane had not been altered by the treatment with nitric acid.

Again, grains were soaked in a 5 per cent. solution of nitric acid, containing 3 per cent. of silver nitrate; they were then rinsed and placed in a 5 per cent. salt solution. From this the grains were rinsed, halved in different planes, and exposed to light. There was no evidence of general penetration; in a few local blackening was evident, but in the majority the exposed surface remained white. Following these experiments, a test was made of the efficiency of the selective capacity of the tissues forming the path of entry after immersion in nitric acid. 150 grm. of barley were steeped in 1 per cent. nitric acid for sixty-eight hours. The grain swelled very considerably. The acid was poured off, and the grains were thoroughly rinsed and stood in a large bulk of tap-water for forty-eight hours with several changes. The last water gave no colour reaction with blue litmus paper. After this the grains were allowed to dry, and they ultimately weighed 148 grm. The acidity of the grain was tested after drying. Some few grains were cut across and steeped in distilled water; the cut surface gave a slight acid reaction with litmus paper. The whole lot of grain was then steeped in 250 c.c. of 4·9 per cent.  $H_2SO_4$ , and maintained at 20° C. The nitric-acid treated barley concentrated the acid solution to the same extent as did a similar weight of normal barley set up as a control. The results are shown in the following table:

#### CONCENTRATION EFFECT OBTAINED BY STEEPING IN $H_2SO_4$ .

Time	Hours.	Hours.	Hours.	Hours.	Hours.	Days.
	0	24	48	72	96	4 <sup>2</sup>
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
' Nitric barley '	7·5	8·1	8·26	8·22	8·12	7·7
Normal "	"	8·14	8·26	—	8·0	7·44
Control "	"	—	7·52	—	7·5	7·5

The figures show the number of c.c. of a solution of KOH required to neutralize 1 c.c. of the acid.

Because the grains gave an acid reaction after drying, the solution of sulphuric acid in which the grains were being steeped was tested after forty-eight hours for nitric acid. Neither by the coloured ring test, with ferrous sulphate nor the starch blueing test in the presence of nascent hydrogen could the presence of nitric acid be detected.<sup>1</sup>

#### (c) *Uptake of stains.*

Additional evidence that penetration took place at a special point or points at the micropylar end was afforded by further immersion experiments. A brief description of these follows.

(a) Grains were steeped in aqueous solutions of methylene blue and safranin in shallow dishes. The coleorhiza frequently broke through the

<sup>1</sup> A slight tint was given in the latter test, but on repeating it, together with a control of the original sulphuric acid, the same slight tint was given in each case.

coverings under these conditions, but hardly any further growth took place. Before the rupture of the grain-coverings occurred, the micropylar point and the furrow tissue at the germinal end became very deeply stained. Cutting round the coverings and lifting the scutellum, the surface opposed to the endosperm showed a gradation of colour which was more intense on the furrow side. The point of the coleorhiza—formed by the embryonic appendage—was deeply stained when it appeared through the coverings. Later, the endospermic bag became very turgid, and burst at the germ end of the furrow. The non-removal of the products of ferment action, owing to the absence of embryo growth, with a consequent rise of turgor pressure, conducted to this result.

Normally it would appear that after the rupture of the coverings at the micropylar end by the development of the coleorhiza, the endospermic bag, bounded by that part of the membrane which remains intact and the scutellum, forms a distinct unit. At this stage the cuticular membrane of the tegmen serves to prevent the loss of the now more or less fluid reserves and to protect this rich pabulum from the attacks of fungi and bacteria.<sup>1</sup>

(b) Grains were steeped for varying periods in a 3 per cent. solution of silver nitrate and the sections cut from them were exposed to light, precautions being taken to guard against the possible diffusion of the solute over the sections. With short periods of steeping the silver nitrate showed no effective entry, beyond a darkening or an occasional blackening of the tissues of the furrow and the cells of the embryonic appendage. After longer periods of steeping the embryo and the proximal end of the endosperm were dark brown, whilst towards the apex of the grain infiltration along the sheaf-like mass of cell-walls and gradual spreading across the section was shown.

The whole series of experiments described in this section represent an endeavour to locate the path of entry of solutes into the grain. From the results obtained, the following general conclusions were drawn:

(a) That, as with water, solutes gained entry at the micropyle.

(b) That in, the earlier stages of immersion of grains in aqueous solutions, the balance between the solute and water was determined solely by the selective action of a tract constituting a specialized local path of entry.

<sup>1</sup> In this connexion my attention has been called recently to the work of J. Beauverie, 'Les Germes de Rouilles dans les Semences de Graminées', Rev. Gén. de Bot., 25 bis, 1914, from which is taken the following extract: 'Dans tous les cas de grains de Graminées contaminés par le mycélium de rouille nous avons pu constater que le dit mycélium ne pénètre jamais ni dans l'albumen, ni dans l'embryon. Il paraît arrêté dans sa progression vers ces organes par la couche membraneuse fortement cutinisée et sclérisée, d'origines diverses, qui recouvre la couche à aleurone dans le cas des fruits nus et par les épidermes contigus de la glumelle et du péricarpe dans le cas des fruits vêtus. Ce n'est que lorsque cette zone membraneuse vient à être rompue par une cause accidentelle, un traumatisme ou peut-être l'action de certaines bactéries, que les bactéries et les mycélium peuvent pénétrer.'

(c) That with longer periods of immersion infiltration of the solute probably occurred through the tissues of the furrow, and that this infiltration took place more rapidly at the germ end.

#### SECTION V. THE SPREAD OF ENZYMES ON GERMINATION AND THE PATH OF LIQUID PENETRATION.

Brown and Morris (2) showed that the germination of the barley grain embryo was accompanied by a definite progression of the disintegration of the endosperm by the action of enzymes. It was stated that the mass of residual cell-walls lying in contact with the scutellum, particularly its upper two-thirds, was the first to be dissolved, whilst the one-third part adjacent to the furrow side of the grain still to some extent retained its structure.

Progress of disintegration proceeded from proximal to distal end, but more rapidly immediately under the aleurone layer. The cells and their contents lying below the corrosion area were then gradually dissolved by the deeper penetration of the enzymes (see Fig. 9). At this time it was sought to prove that the enzymes active in disintegration were wholly produced by the epithelial cells of the scutellum.

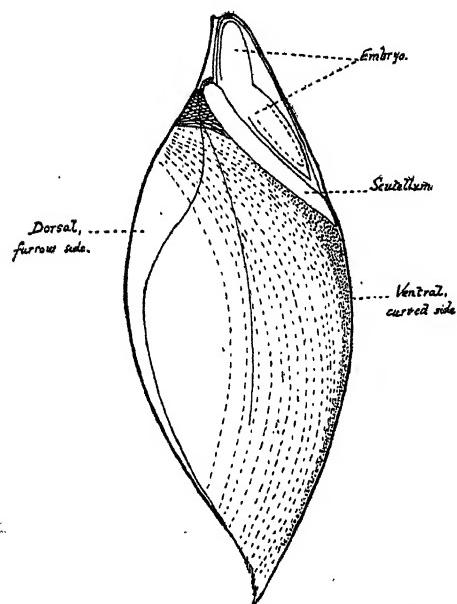
Brown and Morris concluded that the early disintegration of

FIG. 9. Progress of starch disintegration by enzyme action.

Enzyme action first occurs in cross-hatched area, then in dotted area, and subsequently as indicated by broken lines.

the peripheral starch cells was because these cells were younger and their walls less resistant to enzyme action. This explanation cannot be regarded as satisfactory, inasmuch as it is generally understood that the parietal cells of the endosperm are the first to be laid down and provided with walls. An examination of the developing grain of barley showed that this was no exception to the general rule; indeed, the aleurone cells forming the parietal layers possess very thick and highly resistant walls, although they are not cuticularized, as is usually stated.

Haberlandt's (10) account of the progression of enzyme action is, in the writer's opinion, more detailed and exact. He stated that corrosion of the starch granules first occurred between the scutellum and the aleurone layer



on the furrow side. Then the action rapidly extended to the starch granules adjacent to the ventral side of the scutellum. As germination proceeded and the action upon the starch gradually extended, this was found to take place much earlier in the cells of the endosperm lying immediately under the aleurone layer than in those of the central portion of the endosperm, and he observed that dissolution of the starch on the dorsal side in the neighbourhood of the furrow took place last of all. No doubt the rapid disintegration of the sub-aleuronic starch cells led Haberlandt to endeavour to prove that the aleurone layer was active in secretion. He finally came to this conclusion, although he was not able to obtain positive evidence by a direct test with a portion of the aleurone layer itself when isolated from a ripe dry resting grain.

In criticizing Haberlandt's experimental evidence and refuting the theory of aleuronic secretion, Brown and Morris recorded the fact, which is interesting as bearing directly on the argument put forward in the present paper, that there was no evidence of isolated action anywhere along the seat of the aleurone cells such as one would expect if the aleurone cells were capable of secretion.

Since 1890 many papers have been published dealing with the origin and distribution of the enzymes in the barley grain and the related phenomena of life and death of the endosperm and its capacity for self-digestion. These problems have been attacked by various investigators from different points of view.

It will be seen that the spread of enzyme action is precisely the path of penetrant liquids within the endosperm, and there can be no doubt that the phenomena are intimately related. Water undoubtedly enters the grain by way of the micropyle and the embryonic appendage; in this way the needs of the embryo are rapidly supplied. It is also considered probable that, later, water passes to the endosperm at the germinal end through the furrow tissue, which at this point comprises only the glandular group of cells. Was it possible that the water took up the ferment or an activator during the course of its directed passage into the grain? A few preliminary experiments were made in an endeavour to answer this question, and although no absolute conclusion was reached it has been thought as well to place the results on record. Whatever conclusion may ultimately be arrived at, the mode of entry of water into the grain and its distribution in the endosperm makes the mode of progression of enzyme action the more easy to understand.

The embryos were carefully removed from 100 dry grains; from each grain the germinal end of the furrow tissue was cut out, avoiding as far as possible the starch cells. Similarly, small chips of the coverings and subjacent tissues from the curved surface of the grain were cut out. These isolated portions were placed to soak for a short time in a few cubic

centimetres of water contained in watch-glasses. Some thin starch solution was made by boiling a little potato starch in water and diluting it; 20 c.c. were put into each of three small flasks. To A the furrow portions, together with the steeping liquid, were added; to B the portions from the curved surfaces; to C nothing at all. All were maintained at 50° C.

After eight hours 2 c.c. were withdrawn from each flask, and successively tested for sugar by adding three drops of Fehling's solution and boiling. A gave a copious precipitate, as it did even after four hours; B became foggy, and C remained unchanged. After twenty-four hours, A gave an abundant precipitate; B, a very evident one; C, none. Finally, iodine was added to the remaining fluid in each flask; that in A and B gave no starch reaction; that in C did. Whilst, however, the boiled starch solution had been completely converted, the starchy tissue attached to the portions of material in B reacted with iodine. During the experiment the furrow portions had swollen greatly and become soft, while the chips from the curved surface of the grain had swollen but little. The early conversion of the starch to sugar in the first flask might be related to a capacity of the furrow tissue to swell more quickly, and the contents of its cells to diffuse more readily. Considerable quantities of enzyme must, however, be present in the cells of the furrow tissue at its germinal end.

It will clearly be evident from this section that the spread of enzyme action is readily explicable on the theory of the uptake and distribution of penetrant liquids put forward in this paper. Hitherto it has been assumed that, when grains are steeped in water, uniform absorption occurs over the whole surface of the grain, and the effect of a special localized entry with a rapid distribution in the sub-aleuronic starch layer of the curved surface has not been considered. In the opinion of the writer, failure to recognize the local uptake of water and its subsequent peripheral distribution has led in a large measure to the conflicting results which have been obtained by investigators of the problems previously mentioned. Even when embryos or other parts have been excised from the dry resting grain preparatory to experimental work, it is quite possible that a casual wetting during harvesting, or after, may have served to distribute enzymes within the endosperm.

#### SECTION VI. DISCUSSION OF THE POSSIBLE PATHS OF EASY PENETRATION IN THE GERMINAL REGION OF THE GRAIN.

It is now clear that somewhere in the germinal region of the grain there is a path of easy permeability. As it has not been found possible to locate the precise path by direct experimental work, for the adequate reasons previously given, it becomes necessary, in discussing the possible point of entry and seat of selective action, to take into consideration the

facts relating to the structure of the grain and the evidence obtained by the use of penetrant liquids.

Real contact between the tegmen and the embryo is made only at the micropyle by means of the so-called embryonic appendage, and again over the peripheral rim of the scutellum through the agency of a single layer of modified alcurne cells. The tegmen, it must be remembered, possesses a thick cuticle on its outer side and a much thinner one on the inner.

From the evidence obtained experimentally two points of entry suggested themselves, one the micropyle and the other the tract of chalazal tissue in the furrow where it meets the dorsal margin of the scutellum. One regards the micropyle as a natural rapid inlet when seeds are steeped in water. The barley ovule shows a normal open micropyle giving access to the embryo sac, and it can hardly be doubted but that it remains available for the uptake of liquid after the maturation of the grain. Immediately underlying the micropyle is the embryonic appendage, a small mass of cells without apparent contents, which forms the apex of the root-sheath; the mass is fused with the tegmen, and from its position it is assumed that it represents cells which have not been utilized in the formation of the embryo proper. This appendage swells and contracts rapidly when a section of the grain containing it in position is alternately irrigated with water and alcohol. No definite conclusion was reached concerning the chemical nature of the cell-walls of the appendage, but there was some evidence that they were modified in the direction of pectic, mucilaginous, or gum compounds. Such a tissue might act differentially in that water would readily pass, whilst a selective action, possibly adsorptive in nature, would be exercised upon a solute, and its filtration would be rapid, slow, or wellnigh impossible according to the nature of the action of the solute. Moreover, the capacity of the liquid to swell the tissue of the embryonic appendage, or rather perhaps the capacity of the tissue to imbibe the liquid in which the grains are steeped, must be considered in connexion with the question of penetration. Anhydrous liquids are not able to pass into the grain, but in association with water they enter freely. Association with water leads to imbibition and swelling and the subsequent entry of the solutes.

Careful consideration has been given to the possible passage of solutions along the cells of the tegmen, between its inner and outer cuticularized membranes, and the selective action of the inner. The inner cuticle is the less prominent and resistant, and might fail after a time. The embryonic appendage might serve as a temporary hindrance to the passage of solutes into the embryo, whilst the main stream would pass between the cuticularized membranes and be distributed in the distal direction. It should be said here that such a method of distribution assumes that the connecting anticlinal walls between the inner and outer membranes at the

micropyle fail to resist the passage of solutes. In the ovule these walls bounding the micropyle were found to be cuticularized.

As an aid to absorption at the micropyle, the lodicules with their marginal fringe of long hairs serve in the early stages to draw up and hold liquids over the micropylar area.

To turn now to consider the other possible point of entry, the tract of chalazal tissue in the furrow where it meets the dorsal margin of the scutellum. At this point the chalazal tissue, reduced to a group of cells, comes practically flush with the surface of the grain, and once traversed, gives entry to both embryo and endosperm. It has been shown that these chalazal cells form a glandular group. In the mid region of the grain, in addition to this group, there is a mass of closely compressed cell-walls directed towards the centre of the grain. This mass represents the crushed remains of chalazal cells engaged in distributing supplies to the endosperm of the ripening grain. (See Figs. 2, 3, 4, and 6.) As previously mentioned, the nucellar epidermis is absent from the chalazal tract. The mass of cell-walls would presumably hinder the passage of liquid after it had passed the glandular group of cells. That it would further differentiate between solute and solvent is possible, but it is doubtful whether it would prevent all passage of the solute. It seemed clear that solutes were able to pass the glandular cells, for a precipitate of silver chloride was seen at the base of the sheaf-like mass of cell-walls after successive immersion in silver nitrate and sodium chloride for periods of 48 hours. Generally, however, after prolonged steeping in these solutions, there was good evidence of the further penetration of the solutions into the mass of cell-walls, and the arrangement of tissue along the course of the furrow would allow of a much quicker entry at the germinal end. Occasionally a precipitate was seen between the tegmen cuticles on the flanks of the furrow, and it was surmised that the compressed mass of cell-walls offered very considerable resistance to the passage of liquids and the solutions gained entry between the cuticular membranes from the glandular group of cells.

The writer is of the opinion that the phenomena discussed justify the conclusion that the micropyle is the point of rapid entry and the seat of differential action through the agency of the embryonic appendage, whilst slow differential filtration takes place across the chalazal tissue lying along the furrow.

#### CONCLUSIONS.

From the evidence collected in the present paper it is concluded that :

1. Only a small part of the water absorbed by the grain of barley when steeped in various solutions enters by the general surface of the grain, which is invested by three layers of cuticularized cell-wall.
2. Special spots for the entry of water occur in the germinal region

of the grain, and here must be sought the structure which is the seat of the remarkable selective permeability which keeps out mineral acids and most salts whilst passing water with considerable freedom.

3. Solutes such as iodine and acetic acid, which have been recorded as penetrating the grain more or less readily, hardly pass at all through the general surface of the grain, but, like water, enter by the restricted region at the germinal end.

4. The penetration of nitric acid, which also takes place at the germinal end of the grain, is the result of selective action ; the acid neither destroys the enveloping membrane nor impairs the efficiency of the selective structure.

5. The barley grain does not appear to possess perfect impermeability to any solute. Sulphuric acid gradually enters if the grains are kept in the solution for many days. In correlation with this it is observed that the initial concentrating effect of barley upon dilute sulphuric acid gradually falls back, and ultimately the steeping solution becomes even weaker than before it was brought into contact with the grain.

6. It is at the outer cuticularized wall of the tegmen that the recorded arrest of silver nitrate and sodium chloride penetration takes place.

7. This cuticularized membrane is permeable to water and solutes only to the extent usually associated with cuticle. It is possible to construct an osmotic cell by blocking a glass tube with a plug of the grain-covering. If this cell is filled with a salt solution on one side and with water on the other, slow passage of water takes place for months, but no salt passes in the reverse direction until some considerable time has elapsed.

8. The initial uptake of water supplies the need of the embryo ; the grain-coverings form a well-constructed system for ensuring that the supply of water shall be carried along superficially to that part of the grain where it can be readily absorbed by the embryo.

9. The subsequent distribution of liquid in the endosperm is precisely the path of enzyme disintegration within the endosperm during the germination of the embryo. It is suggested that the uptake and distribution of water in germination prepares the way for the distribution of, or the water even takes up and carries with it, the enzymes active in the solution of the reserves.

In conclusion, I desire very cordially to thank Mr. F. F. Blackman, F.R.S., for his interest, advice, and criticism, during the course of this research, which was carried on in the Botany School, Cambridge, 1914.

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# Centrosomes in Fertilization Stages of *Preissia quadrata* (Scop.), Nees.

BY

MARGARET GRAHAM.

With Plate X.

SO far as the evidence in the literature on the subject goes, it seems clear that while centrosomes are present in the nuclear division figures of many algae and fungi, they are just as regularly absent from nuclear division figures in the higher plants. In both, however, they are active as blepharoplasts in connexion with the formation of cilia. There is very little evidence as to whether centrosomes are present during the stages of fertilization in plants, though in animals it is fairly well established that, in many cases at least, the centre which has been brought into the egg by the sperm divides in the formation of the cleavage spindle. In the Bryophytes and Pteridophytes, in a number of cases, the centre which becomes the blepharoplast is described as arising by division from a centre which has appeared at the poles of the karyokinetic figure in the last preceding division or in several of the preceding nuclear divisions. No one has, however, followed the centre through the processes of fertilization in these forms. As is described below, I have been able to demonstrate that in *Preissia quadrata* centres are present at the time when the pronuclei come together.

It is not to be forgotten that evidence has accumulated that structures at least closely simulating centrosomes in appearance can be produced artificially in the cytoplasm of eggs stimulated with chemicals.

As a result of stimulating the egg-cells of *Strongylocentrotus lividus* at various stages, Hertwig (1887) observed artificial astrospheres in the cytoplasm, sometimes isolated and sometimes associated with chromosomes. The astral rays of these artificial astrospheres of Hertwig are said to radiate from a clear area in which there is no centrosome.

By stimulating the eggs with salt solutions Morgan (1899) induced astrosphere-formation also in the cytoplasm of the unfertilized eggs of *Arbacia*. These artificial astrospheres of Morgan centre on a dense body. Morgan also observed many astrospheres with clear centres similar to those

described by Hertwig. And artificial astrospheres (cytasters), some with a clear centre, others with a granule on which rays centre, were observed by Wilson (1901) in the stimulated cytoplasm of unfertilized eggs of *Toxopneustes variegatus*.

Central bodies and asters in the Algae are apparently related to cell and nuclear division in the same fashion as in animals. Radiations were observed by Farmer and Williams (1896-7) at opposite poles of the oogonial nucleus and also from the cleavage nucleus in *Fucus vesiculosus*; and in the germination of the oosphere of the same plant granules that stand at the centre of a ray system were observed by the same workers (1898). Two such systems are described as forming the poles of the fully formed spindle appearing during the prophases and persisting through the anaphases. Strasburger (1896-7) observed in *F. serratus* that two centrosomes with their radiations lie on the nuclear membrane of the fusion nucleus as it enters the anaphases. They lie on the margin of the fusion area of the two sexual nuclei, he points out, and form the poles of the fully formed spindle. Swingle (1897) observed fibres that radiate from definite small points at the poles of the nucleus in *Stylocaulon*. These systems, it is claimed, originate from a granule lying on the nuclear membrane; and kinoplasmic threads from the same centrosomes enter the nuclear cavity, become attached to the chromosomes, and form the spindle. A rod-shaped centrosome on opposite poles of the nucleus and at the centre of diverging rays was observed in *Dictyota dichotoma* by Mottier (1900). Mottier also states that fibres penetrate the nuclear membrane and that some of them become attached to chromosomes.

In the Fungi a relationship that exists between the chromatin and central body in *Phyllactinia corylea* during karyokinesis and the resting period was observed by Harper (1905). In addition to this relation to division Harper observed that centrospheres are concerned with delimiting the mass of cytoplasmic substance about the nucleus of the forming ascospore.

The majority of recent authors, Hirase (1898), Belajeff (1899), Ikeno (1908), Chamberlain (1903), Jahn (1904), and Allen (1912) seem to regard the blepharoplast as a modified central body.

Farmer (1895) has described the centrospheres in the dividing spore mother-cell of *Pellia epiphylla* as arising at four points on the nuclear membrane, points from which radiations extend into the lobe nearest it. A minute centrosome was observed within the centrosphere. It is in the Bryophytes that we may expect to find clues to the explanation of the relations between the method of spindle formation in the higher plants where centrosomes are not present and the method of spindle formation in the Algae, Fungi, and animals where centrosomes are commonly present. There is considerable evidence that centrosomes are present in the vegetative divisions of the cells of Liverworts. In the germinating spore of *Pellia*

*epiphylla* Farmer and Reeves (1894) describe two minute structures on opposite sides of the nucleus in contact with its membrane. Radiations extend from these structures into the cytoplasm and later over the nuclear membrane; and as the latter disappears, the spindle is formed. At opposite poles of the elongated nucleus of cells in the stalk of the archegoniophore of *Marchantia polymorpha* van Hook (1900) observed a body from which radiations extend, some of which penetrate the nuclear membrane. Kinoplasmic radiations in the germinating spore of *Pellia epiphylla* have been described by several investigators, first by Farmer and Reeves (1894). Farmer (1895) described them as radiating either from a minute centrosome or from a group of granules outside of which is a hyaline space. Davis (1901) observed asters that radiate from a vague centrosphere-like region at opposite sides of the nucleus. Chamberlain (1908) observed caps at the poles of the nucleus that become resolved into fibres; and Grégoire and Berghs (1904) describe cytoplasmic fibres oriented on the poles of the nucleus either on a polar vesicle or on the nuclear membrane, which radiate towards the cell membrane or towards the equatorial plane enveloping the nucleus. The aster is described as a cytoplasmic network, the rays of which are joined among themselves. Both centrospheres and centrosomes are said to be absent. In the Mosses Allen (1912) observed plates of kinoplasm that occupy opposite sides of the nucleus of the antheridial cells of *Polytrichum juniperinum*. These kinoplasmic plates are formed by the division of a single plate into two daughter plates, and are connected by fibres. In later divisions the kinoplasmic plates are replaced by bodies, and finally by a central body.

I have studied the process of fertilization in *Preissia quadrata* at a stage when the nucleus of the antherozoid lies near the centre of the egg. My material was collected from gorges around Ithaca and prepared for sectioning at Cornell University. I am indebted to Prof. G. F. Atkinson for the privileges of the botanical laboratory there, where for several years I experimented with methods of handling the plants and with various killing reagents. The material from which drawings were made for this article was killed in the field in a modified Flemming solution. I also acknowledge the privileges of the laboratory in the Cornell Medical College at Ithaca. My material was stained and studied at the botanical laboratory at Columbia University and examined by Prof. R. A. Harper, to whom I am indebted for a critical examination of my preparations.

Fertilization stages in *Riella Clausonis* have been described by Dr. Osvaldo Kruch, who reports that he saw many eggs with one antherozoid in the cytoplasm. Both nuclei, before fusion, were approximately of the same size. The male nucleus is said to contain eight chromosomes, as does also the egg nucleus. Fusion of the pronuclei was not observed, nor were astral rays and centrosomes.

In the present paper I shall describe only the stages after the egg has been penetrated by the antherozoid, when the pronuclei are already near together or in contact (Pl. X, Figs. 1, 3). During these stages the cytoplasm of the egg of *Preissia quadrata* is plainly made up of two zones. The inner zone is granular, with rounded bodies forming a dense aggregate that lies in masses around the pronuclei and among the rays of the centrospheres. A small amount of the same material also clings to the cytoplasmic fibres at the periphery of the cell (Figs. 1, 3, 4). This dense cytoplasm may appear more or less alveolar at this stage. Prior to fertilization the whole cytoplasm of the egg has this consistency and is quite dense. The outer zone of the cytoplasm is coarsely vacuolar. The films between the vacuoles are very thin and delicate. A few larger and quite dense homogeneous granules are scattered through both zones of the cytoplasm. Between the nucleus of the antherozoid and the egg nucleus lies a small mass or body unlike the cytoplasm just described. From its position in close proximity to the nucleus of the antherozoid, the small quantity visible, and the absence of similar cytoplasm anywhere else in the cell, it might be thought to be cytoplasm brought in by the antherozoid, but I have no proof that this is the case. The egg and male nuclei are plainly differentiated by their size and in Fig. 3 lie in the central part of the cell, separated by a very little cytoplasm. In Fig. 1 their membranes are in contact.

In the cytoplasm at the opposite poles of the egg nucleus astral rays are seen converging upon small, dense, rounded bodies, the centrosomes (Figs. 1, 3, 4, 6). These rays extend long distances through the cytoplasmic ground substance. They may pass close to the nuclear membrane or may touch it. A fibre radiating from the centrosome at the upper part of Fig. 1 touches the nuclear membrane of the antherozoid; another radiating from the same centrosome touches the outer membrane of the egg nucleus. These astral rays make up an open aster with few rays; but they are very definite, coarse, granular fibres, easily distinguishable from other cytoplasmic fibres. Peripherally they end rather abruptly and have no conspicuous physical connexion with any of the other elements of the dense cytoplasm. It is quite possible that there are other shorter and more delicate rays, but I have drawn only those which are plainly differentiated. In the fewness of their rays these asters resemble those described and figured by Farmer in the spore mother-cells of *Pellia epiphylla*. Figs. 4 and 6 represent two sections of an egg-cell on either side of the nucleus. The centrosphere shows advantageously in Fig. 4, in which some of the definite, coarse astral rays lie over the nuclear membrane, which is almost free of all other cytoplasmic material, while others extend into the dense alveolar cytoplasm. All these fibres centre on a centrosome, which seems to be a single body. In the section on the other side of the nucleus the centrosphere lies farther away from the nucleus, all of the astral rays running

through dense alveolar cytoplasm (Fig. 6). No well-defined hyaline zone around the centrosome was recognizable at this stage. The region towards which the fibres converge varies in appearance. It has been variously described as a protoplasmic structure of minute size without a centrosome (Farmer and Reeves, 1894); a hyaline sphere containing a centrosome in its centre (Farmer, 1895); a centrosphere-like region of dense protoplasm, the interior of which is frequently granular (Davis, 1901); and a body consisting of the same substance as the astral rays (Chamberlain, 1903). Fig. 1 shows the nuclear membrane of the egg somewhat drawn out to a point where the centrosome lies upon it. The egg nucleus is thus apparently responding to the influence of the centrosphere at one pole. The nucleus of the antherozoid has progressed in the cytoplasm of the egg so that it lies partly beneath the nuclear membrane of the egg nucleus. Two of the rays from the centrosphere nearest it touch its membrane.

Centrosomes and asters are readily demonstrable in the sporophyte of *P. quadrata*. Fig. 7 represents a four-celled embryo. Part of the nucleus and the other centrosphere of each cell are in another section.

From my studies it is evident that centrosomes as definite granular bodies are present not only in the divisions just preceding spermatogenesis and, as blepharoplasts, during metamorphosis, but also in the fertilized egg at the time when the pronuclei are paired. I have further observations soon to be published on the behaviour of the centres during the earlier stages of the fusion of the antherozoid with the egg.

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### EXPLANATION OF FIGURES ON PLATE X.

Illustrating Miss Graham's paper on Centrosomes in Fertilization Stages of *Preissia quadrata*.

The figures were drawn with the aid of a Bausch and Lomb camera lucida, the drawing being at the level of the base of the microscope; Zeiss 1.8 mm. oil-immersion objective, 1.25 N.A., and oc. 4. Magnification about 1431 x.

Fig. 1. Section of the egg-cell. The membranes of the egg and antherozoid nuclei are in contact. Centrospheres lie at the poles of the egg nucleus.

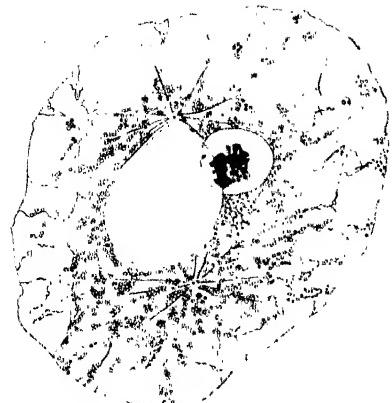
Fig. 2. The contents of the egg nucleus of Fig. 1.

Fig. 3. Section of the egg-cell in which the nucleus of the antherozoid lies in the cytoplasm near the egg nucleus. Centrospheres lie on either side of the egg nucleus.

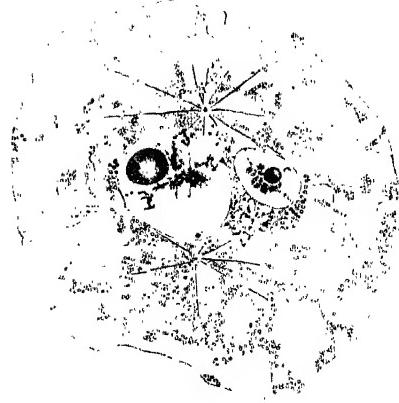
Figs. 4, 6. Polar views of centrospheres lying on either side of the egg, and antherozoid nuclei shown in Fig. 5.

Fig. 5. The egg and antherozoid nuclei situated between the centrospheres represented in Figs. 4, 6.

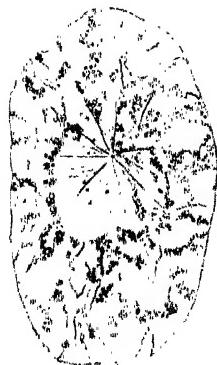
Fig. 7. Two cells of a four-celled embryo. Part of the nucleus and the other centrosphere of each cell are in another section.



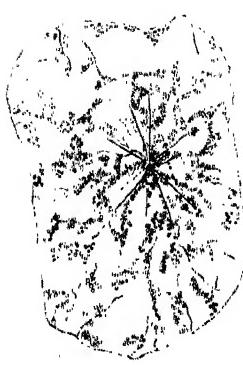
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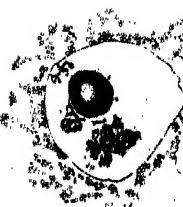
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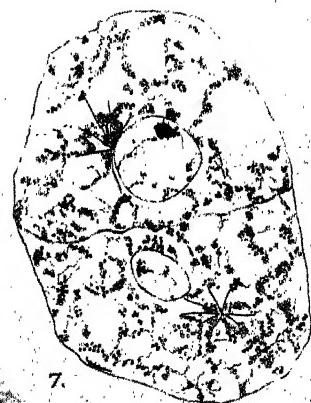
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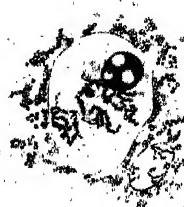
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2.



7.



5.

Hoch, London



# The Genus Caltha in the Southern Hemisphere.

BY

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With ten Figures in the Text.

THE genus *Caltha*, L., was subdivided by de Candolle (Syst., i, p. 307, 1818) into two sections, *Psychrophila*<sup>1</sup> and *Populago*, the distinguishing feature being that in the former the calyx is persistent, while in the latter it is deciduous. Under the section *Psychrophila* he placed two species, *C. appendiculata*, Pers., and *C. sagittata*, Cav., both from Antarctic South America, while the other section included all the Northern Hemisphere species.

It is only with the Southern Hemisphere species that we are concerned in the present paper, and it will be shown that the peculiar foliage characters in these species—which extend from the high Andes of Ecuador to the Falkland Islands, Tasmania, Victoria, and New Zealand—mark them off as a peculiar and remarkable section of the genus more obviously than does the floral character originally noticed by de Candolle.

Berchtold and Presl (Rostl. i. Ranunc., p. 80, 1823) raised de Candolle's section *Psychrophila* to generic rank, which both Gay (Fl. Chil., i, p. 47) and Asa Gray<sup>2</sup> also adopted. Gay gives descriptions of four species, the two additional ones being *P. andicola* (l. c., p. 49), and Hooker's *Caltha dionaeafolia*, described in the London Journal of Botany, vol. ii, p. 306 (Gay, l. c., p. 51).

The general floral similarity of the *Calthas* from the Southern and Northern Hemispheres is so close that the establishment of a separate genus for the Southern forms would prove misleading, and Gay has not been

<sup>1</sup> The name is derived from φυχός, cold, and φύλων.

<sup>2</sup> The following note on the genus *Psychrophila* is given by Asa Gray in Botany U.S. Expl. Expedition, i, p. 13:—“*Psychrophila*: ‘Distinguished from *Caltha* as a genus by the membranaceous appendages which terminate the thickish sepals, either attenuated and as it were caudate, as in *P. appendiculata*, or short and blunt, as in *P. dionaeafolia*; by the few stamens (from 5 to 9); and the few (2 to 9) and 2-8-ovulate ovaries, and 2-3-seeded follicles; the subdioecious flowers; and the very different habit. It must be confessed, however, that the New Zealand species of *Caltha*, recently illustrated by Dr. Hooker, having narrow and attenuated sepals, is too nearly intermediate for the satisfactory discrimination of the two genera.’ In the Genera Plantarum, i, p. 6, *Psychrophila* is maintained as a section of *Caltha*.

*Psychrophila*, Rafin., Atl. Journ. (1832), p. 144, refers to a *Caltha* from Oregon, and his two specific names *P. auriculata* and *P. sagittata* are not synonymous with *Caltha sagittata*, Cav., as the Index Kewensis states, apparently following Torrey in Ann. Lyc. Nat. Hist., New York, ii, p. 164. Rafinesque's plant appears to be *Caltha leptosepala*, DC.

followed by later writers; but it is convenient to relegate the Southern species to a separate section, for which the name *Psychrophila* may be maintained, on account of the peculiar development of the appendages of their leaves.

Eleven species are recognized in the present paper, three being described for the first time. The new species, though collected many years back in the high Andes of Ecuador, Peru, and Bolivia, and in Tasmania, have apparently never received critical examination. The Andine plants have up till now been included under *C. sagittata*, Cav., which is confined to the Magellanic region and the Falkland Islands,<sup>1</sup> and the new Tasmanian species has been placed under *C. introloba* from Australia, from which, however, it is markedly distinct.

The characteristic feature of the plants belonging to the section *Psychrophila*, as now understood, is the development of the auricles of the leaf laminae to form upturned or erect appendages, and this peculiarity is seen in its simplest form in *C. sagittata* and *C. novae-zelandiae*, Hook. f. In these two species the elongated auricles or lobes of the sagittate leaf are sharply folded up from the base, and the appendages thus formed either stand somewhat erect or lie partially over the face of the laminae with their under surfaces facing upwards. In *C. andicola*, Gay, and *C. obtusa*, Chessm., the appendages are so well developed that they are as a rule equal in size to the whole lamina.

Excellent figures of the leaves are given by Cavanilles (Ic., v, Pl. cccxiv), and by Hooker in Bot. Mag., t. 4056, for *C. sagittata*; by Hooker for *C. novae-zelandiae* (Fl. Nov. Zeal., t. 6), and by Gay for *C. andicola* (Atlas Bot., Pl. ii).

The figures interspersed in this paper also illustrate the point, and are included to show the method of development of the more remarkable and distinct appendages of some of the other species. But for the existence of these simple forms of inflexion the morphological significance of the appendages in *C. appendiculata*, Pers. (see Deless., Ic., t. 43), *C. dioneaefolia*, Hook. f. (see Fl. Antarct., ii, Pl. lxxxiv), *C. alata*, A. W. Hill (Figs. 3 and 4), and *C. phylloptera*, A. W. Hill (Fig. 7), would be far from obvious.

The next stage in the development of the appendage from the simple upturned lobe is shown by *C. introloba*, F. Muell. (Fig. 8), and *C. involuta*, A. W. Hill (Fig. 2). In the former species the axis of the fold is no longer at right angles to the petiole, but has become inclined obliquely to that organ at an angle of 45°. The appendages are also considerably elongated towards the apex of the leaf, being produced forward over the surface of the lamina, and, as the fold lies partly open and is not pressed

<sup>1</sup> Urban remarks (Eng. Bot. Jahrb. xxxvii, p. 401) that the genus *Caltha* is represented in collections from Colombia, Ecuador, and Chile by a widely distributed species, *C. sagittata*, Cav. (= *andicola*, Gay). He thus failed to appreciate the morphological peculiarities of the leaves of the Northern Andine forms, and is also incorrect in reducing *C. andicola*, Gay, from Chile to the Magellanic species *C. sagittata*, Cav.

down, the forward free portions of the appendages stand more or less on edge as erect, wing-like organs parallel to the midrib.

In *C. involuta* the inclination of the axis of the fold has been carried a stage farther and has come to lie parallel to the petiole, so that the appendage in this case is a *lateral* fold or flap instead of being a *basal* one as in *C. sagittata*, Cav.

The explanation of the way in which this change in direction of the fold may have occurred would appear to be that the lobes of the leaf have grown basally and peripherally, thus resulting in a curvature of the organ which has carried what was originally a basal infolding through a right angle, so that it has come to lie with the axis of the fold parallel to the petiole and midrib of the leaf.

If, further, the line of attachment of this lateral fold is carried forward, from the lobe of which it is a part, on to the lamina itself, so that it forms a distinct erect wing quite independent of the lobe, the condition represented by *C. alata*, A. W. Hill, from Bolivia, and *C. phylloptera*, A. W. Hill, from Tasmania, will be the result. Here the two wing-like appendages stand erect over the lamina parallel to the midrib and are attached for some two-thirds of their length to the lamina on either side of the midrib (*see Chloris Andina*, ii, Pl. lxxxiii B, under *C. sagittata*, and Figs. 3, 4, and 7).

One of the most remarkable species is *C. dionaeafolia*, which has been so well described and figured by Hooker<sup>1</sup> and by Goebel<sup>2</sup> that little more remains to be said. The two appendages arise from the base of the lamina and are wing-like organs covering the two halves of the lamina. As in *C. alata*, they represent laterally infolded lobes carried up on to the lamina, and their margins, like those of the deeply-bilobed lamina, are bordered with teeth resembling those of the genus *Dionaea* on a minute scale.

The farthest extreme of the appendage development is shown by *C. appendiculata* and *C. limbata*, from the Magellanic region and S. Chile, where the appendages take the form of simple or lobed obovate-spathulate outgrowths from the upper surface of the somewhat similarly shaped laminæ.

#### STRUCTURE OF THE LEAVES.

The leaves of these Southern Calthas show some features of interest in their internal structure, to which attention was first called by Goebel in the case of *C. dionaeafolia*. Here the stomata are confined to the morphologically upper surface of both laminæ and appendages, on which side the feebly developed palisade tissue occurs. Possibly, as Goebel suggests, the closely infolded appendages form an air-chamber, and thus prevent the wetting of the stomata should the leaves of the plant be submerged. The

<sup>1</sup> Hooker, J. D. : London Journ. Bot., vol. ii, p. 306 ; Fl. Antarct., vol. ii, p. 229.

<sup>2</sup> Goebel : Pflanzenbiol. Schild., vol. ii, 1891, p. 27, Fig. 6.

marginal teeth of the lamina and appendages would serve to render such tiny air-chambers more efficient for the exclusion of water.

Diels<sup>1</sup> has further investigated the New Zealand species *C. novae-zelandiae* and some of the S. American species also. He points out that in *C. andicola* a few stomata occur on the morphologically lower side of both lamina and appendage, and that, as might be expected, there is a more or less normal development of spongy tissue. The majority of the stomata, however, occur on the upper surface.

In *C. limbata*, as he points out, the stomata have disappeared completely from the lower surfaces of both lamina and appendage, and the cell walls of the lower epidermis have become considerably thickened—a condition which obtains also in *C. dionaeafolia*—while in *C. novae-zelandiae*, in addition to the thick-walled epidermis, the next layer of cells has also developed thick walls and forms a definite hypoderm which he regards as a special water-storing tissue.

Mr. L. A. Boodle has very kindly examined the leaves of all the

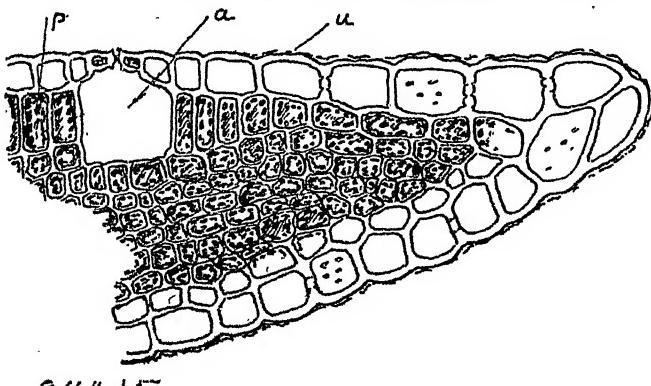


FIG. 1. *C. limbata*, Schlecht. Appendage in transverse section. *u* = upper surface; *p* = palisade; *a* = air-chamber below stoma.

species and finds that in *C. limbata*, *C. appendiculata*, *C. dionaeafolia*, *C. introloba*, *C. phylloptera*, *C. novae-zelandiae*, and *C. obtusa* the stomata are confined to the upper surface of lamina and appendage, and that in all these species the lower epidermis consists of large thick-walled cells and is easily detachable from the rest of the leaf.

In *C. limbata*, *C. appendiculata*, and *C. novae-zelandiae*, especially, the hypoderm cells on the lower side are conspicuously large and have thick walls like those of the epidermis. Both these layers of cells are devoid of chlorophyll and are more or less empty, and in all the species showing this thick-walled epidermis these two layers of cells may serve for water-storage, as Diels suggests. In all these cases normal lacunar spongy parenchyma is absent.

<sup>1</sup> Diels in Engl. Bot. Jahrb., vol. xxii, p. 260. See also Solereder, Syst. Anat. Dicot., vol. i, p. 17 (Eng. trans.).

In *C. sagittata*, *C. andicola*, *C. alata*, and *C. involuta* stomata are found on both surfaces of lamina and appendage, but those on the lower surface are relatively few. Normal spongy parenchyma also occurs and the lower epidermis is not specially differentiated. The stomata are also found to be more abundant on the appendages than on the laminae.

In all these southern species the arrangement of the stomata on the upper surface is very regular and resembles that of parallel-veined leaves, the pores being all set parallel to the midrib.

In *C. limbata*, *C. appendiculata*, and *C. novae-zelandiae*, in particular, the air-chambers beneath the stomata are remarkably large (Fig. 1).

The vascular tissue of both laminae and appendages is usually well developed, and in *C. appendiculata*, *C. novae-zelandiae*, and *C. obtusa* well-marked water-pores occur at intervals along the margins of both organs.

The development of palisade tissue in the Antarctic species is, as might be expected, feeble, and in some cases, as for instance in *C. limbata*, chlorophyll sometimes occurs in the upper epidermis.

In *C. introloba* and *C. phylloptera*, which are mountain plants, there is a fairly well differentiated palisade tissue, and in *C. alata* from the high Andes the palisade is very conspicuous and consists of a double or treble layer of narrow cells.

#### FLORAL STRUCTURE.

The flowers of these Southern forms do not appear to differ in any marked degree from those of the Northern Hemisphere species, the Andine species, and also *C. sagittata* and *C. obtusa*, having the familiar ovate or obovate obtuse perianth-segments and numerous stamens and carpels.

In *C. appendiculata*, *C. introloba*, *C. novae-zelandiae*, and *C. phylloptera* the perianth-segments are linear-lanceolate or ligulate, and terminate in more or less acuminate tips, while in *C. dionaeafolia* and *C. limbata* the perianth-segments are terminated by somewhat thickened pads of tissue. According to Hooker<sup>1</sup> the flowers of *C. appendiculata* are dioecious, and A. Gray<sup>2</sup> refers to this species and also to *C. dionaeafolia* as being subdioecious, but these statements cannot be verified from an examination of herbarium specimens.

The carpels are very numerous in some species, especially in *C. sagittata*, and are usually erect when ripe. In *C. andicola* and especially in *C. novae-zelandiae*, however, they are spreading, and in the latter become almost horizontal.

Whether carpillary nectaries<sup>3</sup> occur in these Antarctic *Calthas* cannot certainly be determined, owing to the difficulty of examining herbarium

<sup>1</sup> Hooker, J. D.: Fl. Antart., vol. ii, p. 228.

<sup>2</sup> Gray, Asa: Botany U.S. Expl. Expedition, vol. i, p. 13. See foot-note to p. 421 of the present paper.

<sup>3</sup> Mueller, H.: Fertilization of Flowers, p. 80, Fig. 27 (Eng. trans.).

material for such delicate structures. Some trace of a nectary like that figured by Mueller is noticeable on the carpels of *C. andicola*, but as the nectaries cannot be clearly recognized in dried specimens of *C. palustris*, the point must be left uncertain.

### KEY TO SPECIES.

Leaves sagittate, or cordate with lobes infolded from the base, the axis of the fold being at right angles to the petiole.

Margins of leaves and folds (appendages) entire or slightly sinuate.

Perianth-segments 8–9, ovate, obtuse . . . . . 4. *C. sagittata*

Perianth-segments 5–7, linear-subulate . . . . . 10. *C. novae-selandiae*

Margins of leaves and appendages crenate.

Perianth-segments 6–8, oblong, obtuse;

leaves about 2 cm. long, 2·5 cm. broad . . . . . 3. *C. andicola*

Perianth-segments 5, oblong, obtuse, or  
subacute; leaves 0·8–1 cm. long and  
broad . . . . .

11. *C. obtusa*

Leaves lanceolate-sagittate; appendages narrowly  
oblong-erect, infolded obliquely; axis of fold at  
an angle of 45° to petiole; leaves 2·5–4 cm. long.

Perianth-segments 5–8, linear-lanceolate . . . . .

9. *C. introloba*

Leaves ovate- or cordate-sagittate; lobes infolded  
laterally with axis of fold more or less parallel to  
the petiole; leaves 1–1·2 cm. long and broad.

Perianth-segments 5, ovate, obtuse . . . . .

1. *C. involuta*

Leaves either cordate-sagittate, elliptic-ovate, or  
sub-rotund and deeply bilobed with marginal  
teeth; appendages erect, wing-like, attached to  
the lamina on either side of the midrib.

Appendages simple. Perianth-segments ovate

2. *C. alata*

Appendages bilobed at the base. Perianth-  
segments linear-lanceolate . . . . .

8. *C. phylloptera*

Appendages elliptic, furnished like the laminae  
with marginal teeth. Perianth-segments ovate  
with thickened tips . . . . .

7. *C. dioneaeifolia*

Leaves obovate-spathulate; appendages spathulate,  
narrowed almost to a petiole, and arising from the  
lamina on either side of the midrib.

Leaves with lateral lobes. Perianth-segments  
linear-lanceolate, twice as long as stamens . . . .

5. *C. appendiculata*

Leaves without lateral lobes. Perianth-seg-  
ments ligulate, about as long as stamens . . . .

6. *C. limbata*

The key is mainly artificial and is based especially on the type of leaf appendage. The species are arranged geographically, commencing with *C. involuta* from Ecuador, and the numbers given in the key refer to the sequence of the descriptions.

1. *C. involuta*, A. W. Hill; species distincta, foliis distincte petiolatis appendiculis e latere auricularum inflexis in longum costae mediae adpositis.

*Herba perennis, humilis, acaulis, glabra, circiter 3-4 cm. alta, radice crasse carnosa. Folia late ovato- vel cordato-sagittata, 1·2-1·5 cm. longa et lata, apice retusa, marginibus paullo sinuatis, auriculis bi-appendiculatis; appendiculae erectae, oblongae, obtusae, 7-9 mm. longae, 3-4 mm. latae, ex auricularum lateribus inflexae vel involutae, in longum costae*

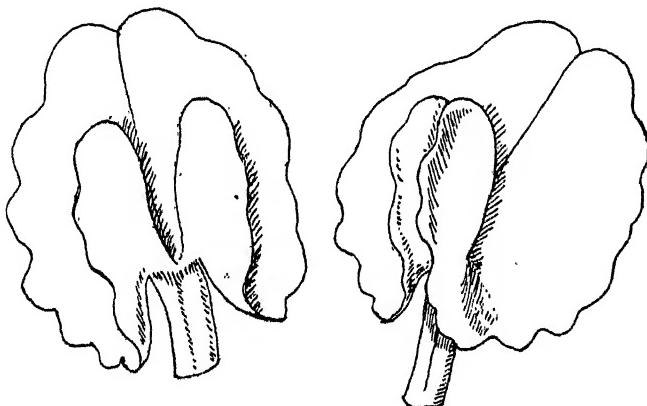


FIG. 2. *C. involuta.* Jameson 457. × 3.

mediae adpositae; petioli circiter 2 cm. longi, ima basi in vaginam amplam dilatati. *Pedunculi uniflori, breves. Perianthii segmenta 5, ovata vel elliptica, obtusa, 8-9 mm. longa, 5-6 mm. lata. Stamina numerosa. Carpella numerosa, in rostro angustata, immatura.*—*C. sagittata*, Wedd., Chl. And., ii, p. 306, et Huth, Abhand. u. Vort. gesammt. Naturwiss., iv, p. 13, quoad spec. Jameson (Ecuador) tantum non Cav. ECUADOR. Cotopaxi, Remy<sup>1</sup> in herb. Mus. Paris; Antisana, 4270 m., Jameson; Cotopaxi, 3,950 m., in damp meadows on Eastern slopes (Fl. March, April), Jameson 457 in herb. Mus. Brit. et Edin.; Quito, Jameson in herb. Mus. Brit.

The erect appendages are formed by the lateral infolding of the leaf-lobes, the axis of the fold being parallel to the petiole. The

<sup>1</sup> M. Jules Remy ascended Chimborazo with Mr. Brenchley in 1856. An account of this ascent, with some remarks on the flora, is given in the Kew Journal of Botany, vol. ix, 1857, p. 143.

appendage is further carried forward in its free apical portion over the lamina, and forms an erect, wing-like organ.<sup>1</sup>

2. *C. alata*, A. W. Hill, species insignis, foliis parvis appendiculis vel aliis oblongis obtusis erectis utrinsecus in longum costae mediae exortis.

*Herba* perennis, humilis, acaulis, glabra, circiter 2 cm. alta, radice elongata carnosa. *Folia* breviter cordato-sagittata, 6–8 mm. longa et lata, obtusa vel retusa, marginibus plus minusve sinuatis, bi-appendiculata vel potius bi-alata; appendiculae vel alae erectae, oblongae, obtusae, utrinsecus in longum costae mediae exortae; petioli circiter 1 cm. longi, basi in vaginam amplam dilatati. *Pedunculi* uniflori, crassi, circiter 1·5 cm. longi. *Perianthii segmenta* 5, ovata vel elliptica, obtusa, flava. *Stamina* numerosa. *Carpella* plurima, curvata, erecta, in rostro

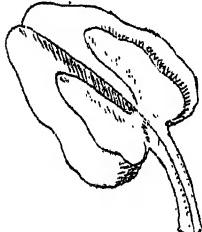


FIG. 3. *C. alata*. Mandon 884. × 3.

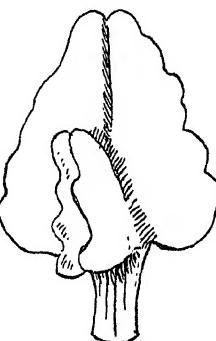
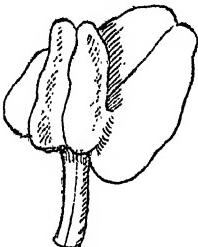


FIG. 4. *C. alata*.  
Weddell 4483. × 3.

acuto attenuata, plus minusve 6 mm. longa. *Semina* circiter 6, atro-fusca, dorso rotundata.—*C. sagittata*, Schlecht. in Linnaea, xxvii, p. 557, non Cav.; Wedd., Chl. And., ii, p. 306, quoad spec. Peruv. et Boliv. tantum et figs., Pl. lxxxiii B, non Cav. vel Gay; Huth, Abhand. u. Vort. gesammt. Naturwiss., iv, p. 13, quoad spec. Peruv. et Boliv. non Cav.

PERU. Prov. Carabaya (June, July, 1847), H. A. Weddell 4483 in herb. Mus. Paris; Ayapata, Lechler 1953 in herb. Paris, partim in herb. Kew.<sup>2</sup>

BOLIVIA. Andes of La Paz (1855), Mandon 134 in herb. Mus. Paris; Dept. La Paz: between Coroico and Lancha; in marshes, 5,000 m. April–May, 1857, Mandon 884 in herb. Paris et Kew.

Monsieur Gagnepain, who has most kindly examined the specimens preserved in the Muséum d'Histoire Naturelle, Paris, and furnished me with valuable information, considers Mandon's specimens, no. 134, are

<sup>1</sup> The drawings of the leaves have been made by Miss M. Smith.

<sup>2</sup> There are two specimens over Lechler's label at Kew, one of which exactly resembles Mandon 884, but the other is quite a large plant very similar to specimens from S. Chile.

the plants from which the very beautiful plate in the *Chloris Andina* (*C. sagittata*) was prepared. The figures show the shape and line of attachment of the wing-like appendages remarkably well. The appendages are attached to the lamina on either side of the midrib, all trace of their being derivatives of the infolded basal leaf-lobes having been lost.

Weddell remarks that this species is very abundant in Peru and Bolivia, where, with *Crantzia lineata* var. *subulata*, it forms a very compact turf and is almost always covered with water.

*C. phylloptera*, A. W. Hill, from Tasmania, is the only other species with similar wing-like appendages, but they differ from those of *C. alata* in having a small detached lobe at the base.

3. *C. andicola*,<sup>1</sup> Walp., Ann. Bot. Syst., i, p. 12; Reiche, Fl. Chil., i, p. 24.

*C. de Ranco*, Steud. in Flora, 1856, p. 407.

*C. sagittata*, Wedd., Chl. And., ii, p. 306, et

Huth, Abhand. u. Vort. gesammt. Naturwiss.,

iv, p. 14, Pl. i, Fig. 1, quoad spec. Chilens.

tantum; *C. sagittata*, Poeppig, Frag. Syn. Pl.,

p. 29. *Psychrophila andicola*, Gay, Fl. Chil.,

i, p. 49; Atlas Bot., t. ii.

CHILE. Rivulets E. side of Andes near Volcano Peteroa, Bridges 1248; Cord. de Ranco, 1,680 m., Pearce 13; Lechler 2981; Colchagua, R. A. Philippi; Baños de Chillan, Reed; Coquimbo, Cord. de Da. Rosa, Volckmann; Cesso Macha, F. Philippi; Antuco, 2750 m., Poeppig; S. Chile, Miers in herb. Mus. Brit.; Gay in herb. Mus. Paris.

According to Gay the native name of this plant is Maillico, and the inhabitants of Colchagua use the root for stomachic affections. Its value in this respect is also mentioned by Reed (Hist. Nat. Chil., p. 97), who states that it is much appreciated for certain infirmities of the stomach.

The plant is beautifully figured by Gay in his Atlas. According to his description and figure the white flowers have 6 petals—Reiche says 5–6—but some of the specimens at Kew have as many as eight elliptic-ovate acute segments. The leaf lobes, infolded from the base, are crenately lobed, sometimes dentate-crenate, and the line of attachment of the appendages is quite narrow, so that they have almost the

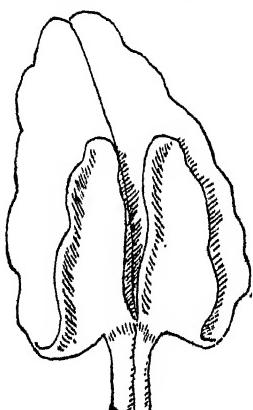


FIG. 5. *C. andicola*.  
Pearce 13.  $\times 3$ .

<sup>1</sup> The combination *C. andicola* is attributed to Gay in the Index Kewensis, and also by other authors, but in Fl. Chil. (l. c.) he places this and all the other S. American *Calthas* under the genus *Psychrophila*. The proper authority for the combination *Caltha andicola* is Walpers, as given above.

appearance of separate organs nearly as long as the lamina. According to Gay's figure the ripe carpels are spreading and not erect as in *C. sagittata*, Cav.

4. *C. sagittata*, Cav., Ic., v, p. 456, t. 414; DC., Syst., p. 307; DC., Prodr., i, p. 44; Gaud. in Ann. Sc. Nat., Bot., v, p. 105, et in Freyc., Voy. Bot., p. 136; D'Urv. in Mém. Soc. Linn. Paris, iv, p. 615; Hook. f. in Bot. Mag., t. 4056; Hook. f., Flor. Antarct., ii, p. 228; N. Alboff, Contrib. Fl. Terre de Feu, ii, p. 9, partim; Exp. Ant. Belg. 'Belgica', p. 88; (et? var.  $\beta$  vegetior) Wedd., Chl. And., ii, p. 306, et Huth, Abhand. u. Vort. gesammt. Naturwiss., iv, p. 13, quoad ref. Iles Malouines et Ter. Mag. tantum; Gray in Bot. U.S. Expl. Exp., i, p. 12, excl. syn., *C. andicola*; Reiche, Fl. Chile, i, p. 25; non Poeppig, Frag. Syn. Pl., p. 29; non Schlecht in Linnaea, xxvii, p. 557; non Torr. in Ann. Lyc. Nat. Hist. N. Y., ii, p. 164 (= *C. leptosepala*). *C. sagittata* var. *latifolia*, Huth, Abhand. u. Vort. gesammt. Naturwiss., iv, p. 14, Pl. I, Fig. 2. *C. multicapsularis*, Soland. MSS. in Bibl. Banks; Forster in Trans. Linn. Soc., viii, p. 324. *Psychrophila sagittata*, Bercht. & Presl, Rostl., i, Ranunc., p. 80; Gay, Fl. Chil., i, p. 47; non *Psychrophila sagittata*, Rafin., Atl. Journ., p. 144, nec *P. auriculata*, Rafin. l. c.
- S. CHILE. Tierra del Fuego, Banks and Solander in herb. Mus. Brit.; Magellan Straits, Sandy Point, Lechler 110; Port Famine, Capt. King 1027; Gregory Bay, R. O. Cunningham; Oozy Harbour (*Nassau Expedition*); Hermite Island, J. D. Hooker, 31; Orange Harbour, Wilkes (*U.S. Expl. Expedition*); Port Famine, Anderson; Fuegia, F. Philippi.

FALKLAND ISLANDS. J. D. Hooker; W. Arnott; R. O. Cunningham; Mrs. Vallentin 104.

This species was cultivated at Kew in 1842. The plants were brought from the Falkland Islands and provided the material for the plate (t. 4056) in the Botanical Magazine. The carpels when young were found to be slightly hairy.

*C. sagittata*, which is now found to be confined to the Falkland Islands and the southern Magellanic Region, has formerly been considered to extend along the Andes to Ecuador, the northern limit of the *Psychrophila* section of the genus. Weddell includes the specimens collected in Peru, Bolivia, and Ecuador, and Schlechtendal also placed Lechler's Peruvian plant under this species. *C. sagittata* is distinguished not only by its size, being by far the largest of the S. American Calthas, but also by the leaves, which have the large auricles folded up from the base, and by the flowers, which have some eight to ten yellow perianth-segments.

The other species with similarly upturned auricles are *C. andicola*,

from S. Chile, with smaller, more reniform leaves with crenate margins, relatively large elliptic-obtuse crenately lobed appendages, and white flowers; *C. obtusa*, Chesse., from the high mountains of New Zealand (see p. 411), and *C. novae-zelandiae*, Hook. f. This last in leaf-form is very similar to *C. sagittata*, and in some specimens the portion of the auricle bent up is quite small, but in its flowers, with their five long linear-lanceolate perianth-segments and the few spreading carpels, it differs very markedly from *C. sagittata*, with its crowded head of erect carpels and ovate perianth-segments. Gay (Fl. Chil., i, p. 50) believes that *C. sagittata*, Cav., is confined to the Magellanic region, and suggests that Poeppig's *C. sagittata* is really his *Psychrophila andicola*; but he does not reduce it. Alboff, l.c., also tries to confuse these two species. Hooker in Fl. Antarct., ii, p. 228, refers to 'a small state gathered by Mr. Bridges in Chile', which again is *C. andicola*.

5. *C. appendiculata*, Pers., Syn. Pl., ii, p. 107; DC., Syst., i, p. 307; DC., Prod., i, p. 44; Deless., Ic., p. 12, t. 43; Gaud. in Ann. Sc. Nat., Bot., v, p. 105, et in Freyc., Voy. Bot., p. 135; D'Urv. in Mém. Soc. Linn. Paris, iv, p. 615; Hook. f., Fl. Antarct., ii, p. 228; N. Alboff, Contrib. Fl. Terre de Feu, ii, p. 9; Exp. Ant. Belg. 'Belgica', pp. 15, 87; Huth, Abhand. u. Vort. gesammt. Naturwiss., iv, p. 14, Pl. i, Fig. 3; Reiche, Fl. Chil., i, p. 25. *C. paradoxa*, Sol. MSS. in Bibl. Banks; Forst., Trans. Linn. Soc., viii, p. 34. *C. holophylla*, Leybold, Ann. Univ. Santiago, 1859, p. 678, teste Reiche, l. c. *Psychrophila appendiculata*, Bercht. & Presl., Rostl., i, Ranunc., p. 80; Gay, Fl. Chil., i, p. 41; A. Gray in Bot. U.S. Expl. Exp., i, p. 13.

S. CHILE. Tierra del Fuego, Banks and Solander; Hermite Island, J. D. Hooker, 17; Orange Harbour, Wilkes (U.S. Expl. Expedition); Port Grappler, Cunningham; Fuegia, Wyville Thomson (Challenger Expedition); Cape Horn, Arnott.

STAATEN ISLAND. Herb. Soc. Hort.

FALKLAND ISLANDS. Gaudichaud; Hooker 66; Havers in herb. Mus. Brit.

According to Hooker the flowers in this species are dioecious, but from the herbarium specimens it is not possible to confirm or refute this statement, though the flowers show both carpels and stamens which appear to be normally developed. The specimens at Kew from

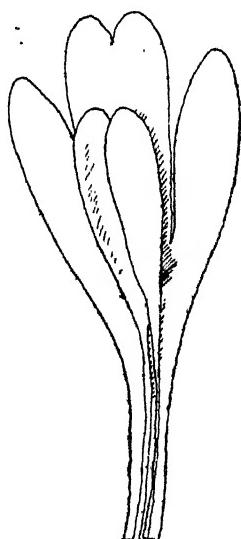


FIG. 6. *C. appendiculata*.

Hermite Island agree exactly with the plant figured by Delessert, with lobed laminae and long perianth-segments tapering to acuminate tips. Some of the specimens from the Falklands and Orange Harbour, however, have simple leaves like those described for *C. limbata*, and the floral difference would appear to be the only essential character for distinguishing these two described species.

6. *C. limbata*, Schlecht. in Linnaea, xxvii, p. 536; Reiche, Fl. Chil., i, p. 25. *C. appendiculata*  $\beta$  *chilensis*, Huth, Abhand. u. Vort. gesammt. Naturwiss., iv, p. 15, Pl. i, Fig. 4. *C. holophylla*, Leybold, Anal. Univ. Santiago, 1859, p. 678 (?).  
S. CHILE. Lechler 3041 (? 3040); Maule, Chillan, fide Reiche, l. c.; without precise locality, R. Pearce in herb. Mus. Brit.

It is doubtful whether this is little more than a variety of *C. appendiculata*, Pers. At Kew and at the British Museum there are three Lechler specimens both bearing the number 3040. Two of them have large simple leaves 4–6 cm. long, and simple appendages with remains of mature fruits, but no flowers, and the locality is given 'Cord. de Ranco ad Seaturez Sichahue, Dec. 54'. The other is a small plant, the leaves being about 2 cm. long, and the appendages are bifid. This is also a flowering specimen and the perianth-segments are ligulate or slightly obovate-spathulate and not much longer than the stamens. They are also terminated in a white membranous rounded tip as Schlechtendal describes. In *C. appendiculata*, Pers., the perianth-segments are twice as long as the anthers and are linear-lanceolate, becoming acuminate at the apex.

As the Kew specimens agree so closely with the description in Linnaea, xxvii, it seems probable that the Lechler no. 3041 quoted is a mistake for 3040.

7. *C. dioneaefolia*, Hook. f. in Lond. Journ. Bot. ii, p. 306; Fl. Antarct., ii, p. 229, t. lxxxiv; Walp., Ann. Bot. Syst., i, p. 11; N. Alboff, Contrib. Fl. Terre de Feu, ii, p. 9; Huth, Abhand. u. Vort. gesammt. Naturwiss., iv, p. 15; Exp. Ant. Belg. 'Belgica', pp. 15, 87, Pl. xx, Figs. 13–18; Reiche, Fl. Chil., i, p. 25; Goebel, Pflanzenbiol. Schild., ii, p. 27, Fig. 6. *Psychrophila dioneaefolia*, Gay, Fl. Chil., i, p. 51; A. Gray in Bot. U.S. Expl. Exp., i, p. 14.

- S. CHILE. Fuegia, Forster and *C. Darwin*; Hermite Island, J. D. Hooker, 15; Cape Horn, Arnott; Davis; Orange Harbour, Wilkes (U.S. Expl. Expedition); Fuegia, Forster; Playa Paeda Cone, Cunningham; Magellan Straits, Mc Whinney; Smith Canal, F. Philippi.

This species and *C. appendiculata* constitute a material portion of the bog-earth of Hermite Island and sometimes cover the ground in broad hard green tufts. The stomatal arrangement has already been

referred to, and though the leaves are so similar in appearance to those of *Dionaea* it has been found that they have no insectivorous function (see Goëbel, l. c.).

8. *C. phylloptera*, A. W. Hill; species cum *C. introloba*, F. Muell., confusa, appendiculis foliorum erectis bilobatis utrinsecus in longum costae mediae exortis distincta.

*Planta* pusilla, acaulis, glabra, 2–4 cm. alta, radicibus carnosis. *Folia* elliptico-ovata, 0·9–1·2 cm. longa, 5–6 mm. lata, apice late obtusa, emarginata, ad basin paulo cordata; appendiculae aliformae, ellipticæ, obtusæ, erectæ, 5–6 mm. longæ, versus basin lobula libera parva instructæ, utrinsecus in longum costæ mediae exortæ; petioli 1–2·5 cm. longi, ima basi in vaginam amplam dilatati. *Flores* singuli, pedunculis 0·5–1·5 cm. longis. *Perianthii segmenta* 5, linear-lanceolata, sensim

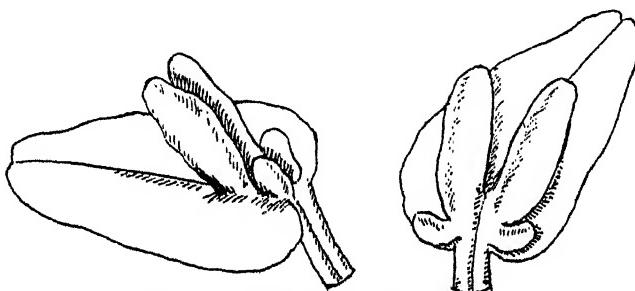


FIG. 7. *C. phylloptera*. Archer. × 3.

acuminata, plus minusve 8 mm. longa. *Antheræa* circiter 10, ovatae, 0·5 mm. longæ; filamenta 5 mm. longa. *Carpella* 6–7, glabra, paulo repanda, rostris 1–1·5 mm. longis rectis instructa. *Semina* 5–6.—*C. introloba*, Hook. f. in Fl. Tasm., ii, p. 355, et Benth., Fl. Austral., i, p. 15, quoad spec. Tasm. *C. novae-zelandiae*, Rodway, Tasm. Fl. (1903), p. 3.

TASMANIA. Western Mts., Archer.

The Tasmanian *Caltha* is a markedly distinct plant from the species found in the Victorian Alps, especially as regards the size and morphology of the leaves. In *C. introloba* the appendages are merely the infolded elongated basal lobes, but in *C. phylloptera* the type of appendage closely resembles that of *C. alata*, A. W. Hill, and as in that species the appendages are entirely detached from the leaf lobe and spring from the lamina itself, forming two erect wings on either side of the midrib. The appendages are usually so deeply lobed at the base that they come to be divided into two very unequal portions.

Hooker, in the Flora of Tasmania, points to the similarity between the New Zealand and Tasmanian forms, and doubts if they are distinct;

but in his comparison he is confusing the Australian, the New Zealand, and the Tasmanian species, and clearly has not appreciated the essential differences in the characters afforded by their leaves.

9. *C. introloba*, *F. Muell.* in Trans. Phil. Soc. Vict., i, p. 98; Pl. Vict., i, p. 10; Hook., Journ. Bot. (1855), vii, p. 234; Hook. f. in Fl. Tasm., ii, p. 355, et Benth., Fl. Austral., i, p. 15, quoad spec. Austral. tantum; Mueller, Syst. Census Aust. Pl., p. 1; 2nd Census, p. 3; Key to Syst. Vict. Pl., ii, p. 5; ibid., i, p. 121; Maiden and Betche, Census N.S.W. Pl. (1916), p. 78. *C. novae-zelandiae*,  $\beta$  *introloba*, Huth, Abhand. u. Vort. gesammt. Naturwiss., iv (1891), p. 15.

AUSTRALIA. Victoria; Haidingor Range, 1370–1680 m., *Mueller*; Mt. La Trobe, *Mueller*. ‘On gravelly places in Australian Alps irrigated during summer by melting snow.’

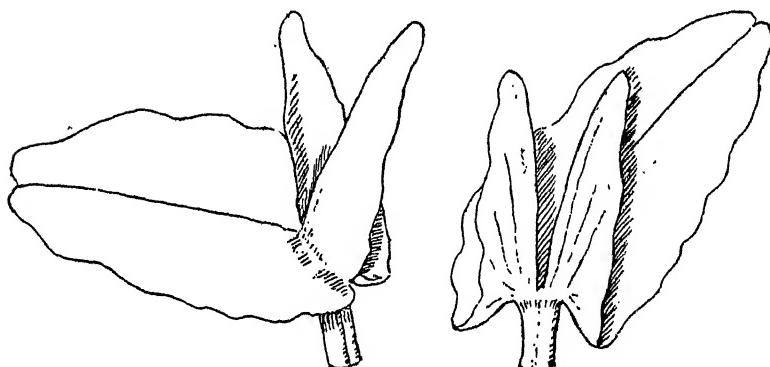


FIG. 8. *C. introloba*, *Mueller*.  $\times 3$ .

The leaves and the appendages, as Mueller points out, are longer and narrower than those of *C. novae-zelandiae*. With regard to the appendages, however, the axis of the fold, instead of being at right angles to the petiole as it is in the New Zealand plant, is inclined at an angle of  $45^\circ$  to the petiole, so that the base of the leaf lobe—one side of which is the folded edge of the appendage—is sagittate. The conspicuous appendages are lanceolate-obtuse, and some 2 cm. or more in length. According to Mueller the whole leaf with its upturned lobes keeps the surface of the leaf away from the icy water in which the lower part of the plant is immersed.

10. *C. novae-zelandiae*, Hook. f. in Fl. Nov. Zel., i, p. 12, t. 6; Hook. f., Handb. New Zeal. Fl., p. 9; Kirk, Students' Flora, p. 21; Cheeseman, Manual New Zeal. Flora, p. 28; Huth, Abhand. u. Vort. gesammt. Naturwiss., iv (1891), p. 15; Featon, Art Album, N.Z. Fl., i, p. 10, Pl. v, Fig. 2. *C. marginata*, Col. in Trans. New Zeal. Inst., xxiii (1891), p. 382.

NEW ZEALAND. North Island to Stewart Island, 750–1,670 m.; various collectors.

The leaf margins are entire and the appendages small. They are formed by the inflexed bases of the lobes of the lamina, the axis of the fold being, as in *C. sagittata*, Cav., at right angles to the petiole. The five perianth-segments are linear-subulate, tapering from the base to almost caudate points, and are yellow in colour.

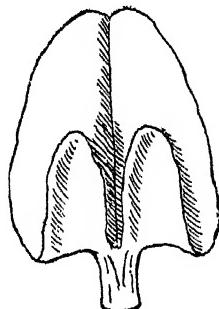


FIG. 9. *C. novae-zelandiae*. Colenso 2336.  $\times 3$ .

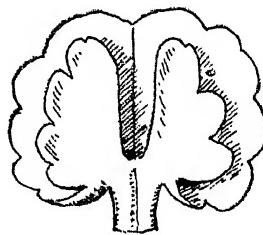


FIG. 10. *C. obtusa*, Hector.  $\times 3$ .

11. *C. obtusa*, Cheesm. in Trans. New Zeal. Inst., xxxiii (1901), p. 312; Man, New Zeal. Fl., p. 28.

NEW ZEALAND. North Island (?), *herb.* Colenso; South Island, mountains, 1,525–2,130 m., *Hector* in *herb.* Kew.

A very distinct species, with its deeply crenately-lobed leaves and appendages. The appendages are formed by the folding upwards of the lobes of the lamina, and are nearly as long and as broad as that organ. The five perianth-segments are white, oblong-obtuse or subacute, and broadest above the middle.



# The Structure of *Mesoxylon multirame*.

BY

D. H. SCOTT, F.R.S.

With Plates XI-XIV and two Figures in the Text.

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In continuation of the description of the Coal Measure genus *Mesoxylon* (Scott and Maslen, 1910; Maslen, 1911; Scott, 1912) an account is given in the present communication of the species *M. multirame*, one of those briefly characterized in the Preliminary Note of 1910. It will be remembered that the genus *Mesoxylon* differs essentially from *Cordaites* in the presence of centripetal wood in the stem.

## THE TYPE.

Several specimens of the species now to be described, *Mesoxylon multirame*, are known. In the first instance I propose to deal with the type-specimen, on which the short description in the Note by Mr. Maslen and myself (1910) was based; this was received from Mr. Lomax in April, 1907. There are 15 transverse and 6 longitudinal sections (Nos. 2329-2343 and 2344-2349 in my collection). The transverse series is remarkably good for following the course of the leaf-traces and axillary steles; the latter accompany the great majority of the leaf-traces.<sup>1</sup>

The diameter of the stem is about 2·5-2·7 cm., that of the pith about 1·2·5 mm. The stem is a rather young one, the wood only reaching a thickness of about 2·5 mm. and the phloem and pericycle together about 1 mm. The cortex is from 3 to 3·5 mm. thick. The leaf-traces are numerous and succeed one another very rapidly, so that two or three are often

<sup>1</sup> The true order of the transverse sections, from above downwards, follows the numbers with two exceptions—2335 comes above 2334 and 2342 above 2341.

cut in a transverse section almost at the same point of their course. That the leaf-bases were fairly crowded is shown by the tangential section 2344 (Pl. XI, Fig. 6). Twenty-six traces were actually observed in the transverse series, representing less than two inches of stem; the true number in this length was obviously greater, as the first three sections are very incomplete. It has not been possible to determine the phyllotaxis in this specimen; the number of orthostichies, provisionally estimated at thirteen in the Preliminary Note, was no doubt higher—possibly twenty-one, though the observed divergences do not agree with an  $\frac{8}{21}$  phyllotaxis.

*M. multirame*, like *M. poroxyloides*, is derived from the ordinary coal-balls or seam-nodules, occurring in the actual coal. The preservation of the type-specimen is decidedly good, though not equal to that of another specimen, since discovered, which will be referred to later.

The pith has the usual discoid structure with a persistent outer zone. The twin bundles of the leaf-trace, unlike those of *M. poroxyloides*, do not fuse immediately on reaching the edge of the pith, but run down side by side for some distance; fusion, in so far as it takes place, is a less simple process. The centripetal xylem is fairly well developed as long as the trace remains double, but disappears about at the level where the strands become merged. The secondary wood, phloem, and pericycle agree very nearly with those of *M. poroxyloides*. The outer cortex has the usual *Dictyoxylon* structure, which in the type-specimen is very well differentiated. The subdivision of the leaf-trace, in passing through the cortex, into eight bundles is clearly shown.

A great feature of the species is the presence of axillary shoots. In the type-specimen they are present in connexion with 19 out of the 26 leaf-traces observed in the transverse series; they appear to be absent from the 7 traces towards the top of the piece of stem, so they were no doubt definitely localized. Their peculiar structure, very different from that of the axillary buds of *M. Sutcliffii*, will be described below (p. 442).

All the best-preserved specimens of *M. multirame* show axillary shoots, but this cannot be used as a specific character, for we cannot doubt that if more material of *M. Lomaxii* and *M. poroxyloides* were available, these species also would prove to have branched in a similar manner.

#### *The Pith.*

In the type-specimen the pith is only moderately well preserved; it is much more perfect in the large stem to be described later. The structure is of the ordinary discoid type (Pl. XIII, Fig. 17), not compound, as observed in *M. poroxyloides*. The more internal cells of the persistent zone, so far as they are preserved, appear nearly empty, while those of the outer part are filled with dense, dark-brown cell-contents (Pl. XI, Figs. 2, 5). Most of the cells are more or less elongated vertically, and this is especially the case

with the outermost pith-cells which border on the wood. These cells, like their neighbours, usually have dark contents. The cells of the diaphragms are much larger and have a different shape, their greater diameter being horizontal. In the middle part of the diaphragms the cells have collapsed and their walls are often torn.

As usual the diaphragms became much thicker towards the periphery. In this case, however, there is often a constriction between the thickest part of the diaphragm and its abutment on the persistent outer zone. Two adjacent diaphragms sometimes unite in this region—a slight approach to the compound condition found in *M. poroxyloides*. There is no important difference in pith-structure between *M. multirame* and *M. Sutcliffii*.

#### *The Leaf-traces.*

The most valuable feature of the type-specimen is the clear way in which the leaf-traces are shown at all points of their course. In a good transverse section, such as that shown in Pl. XI, Fig. 1, many traces can be seen in various positions; but probably the best way to deal with the subject of their course will be to take some one trace and follow it downwards through a series of transverse sections. The illustrations, however, are from different traces, selected as showing the structure best at the various levels figured, for of course we cannot expect the same trace to be equally favourable at all points of its course.

We will take the trace which is numbered 13 in the diagram, Pl. XIII, Fig. 16.

This first appears in section 2335.

Naturally the axillary shoot precedes the subtending trace in approaching the stem and is alone seen in this section (Pl. XI, Fig. 2). The shoot is here partly attached to the cortex of the main stem. It shows the usual extremely flattened stele and parenchymatous cortex with no visible appendages. Three such steles are shown, as it happens, in this section—they are all represented in the photograph, Pl. XI, Fig. 2, and the best of them more in detail in the drawing, Pl. XIII, Fig. 22. The stele measures about 3.5 mm. in length (tangential to main stem) by scarcely 0.25 mm. in width.

In the next section below (2334) (diagram, Pl. XIII, Fig. 16), the axillary shoot has completely joined on to the stem and its stele is seen about half-way through the cortex. The subtending leaf-base has now appeared (though badly preserved) to the exterior of the axillary stele, and at least 7 of the 8 bundles of the leaf-trace are recognizable (see diagram, Pl. XIII, Fig. 16, and cf. Fig. 18). The axillary stele is here less flattened, its dimensions being about 2.5 × 0.75 mm. (cf. the detailed drawing of another stele in Fig. 21).

Proceeding downwards, section 2336 shows the axillary stele (here little more than 1.5 mm. in tangential diameter) in the phloem-zone, but its

adaxial elements are oblique and in the act of joining those of the wood of the main stem. The bundles of the subtending leaf-trace are seen in the cortex, outside the periderm; not all are preserved (five are visible), and it is possible that some fusion among the original eight had already occurred. A somewhat similar stage, but rather lower down, is shown, from another trace, in the photograph, Pl. XI, Fig. 3. In the latter case the leaf-trace bundles have already united so far as to form two symmetrical pairs. In both instances the lateral connexion of the wood of the axillary stele with the stem-wood on either side is well shown.

In the next lower section (2337) the trace, No. 13, has passed within the periderm, and is entering the phloem. Its strands have now united into the pair of bundles characteristic of the trace in the lower part of its course (cf. Pl. XI, Fig. 4, from another trace). The stem-wood behind the leaf-trace is still disturbed, owing to its junction with that of the axillary stele. A pith-bay intrudes into the wood at this level, and a definite median strand of xylem lies directly below the attachment of the axillary stele.

In the next section below (2338) the double trace is just entering the secondary wood, the twin bundles passing in on either side of the median strand, with which they are connected by oblique elements.

One section lower (2339) the twin bundles, converging slightly, have almost reached the pith. There is a space between them, almost equal to the width of a bundle, which is still occupied by the median strand. Almost the same condition, from another trace, is shown from a photograph in Pl. XI, Fig. 5. The bundles at this level have well-developed centripetal xylem; there is no definite sheath, though sometimes a few short tracheides are found in the tissue on the inner side of the bundles.

At the next lower level (2340) the double trace has definitely taken up its position on the inner edge of the wood, bordering on the pith, into which the two strands project somewhat. They are nearer together than before, and the median strand between them is no longer well marked; it stops short of the pith. The bundles of the trace retain their centripetal wood with little if any diminution. A similar stage is shown in Pl. XIII, Fig. 19.

One section below again (2342) the trace still forms a prominent pair; the twin bundles are quite distinct but approximated; near the pith a biserrate ray separates them. The median strand has split up, and its tracheal rows on either side have joined the wood of the twin strands, from which they can no longer be distinguished. The position of the protoxylem is difficult to make out; but the centripetal wood is evidently much reduced, nearly the whole xylem belonging to the radial centrifugal series.

The next section (2341) shows an interesting change. The two bundles of the trace are no longer distinct; the trace is here represented by a single broad xylem-mass projecting into the pith. The mass is made up of seven radial bands of tracheides, separated by medullary rays. There is no longer

any distinction between the trace-bundles and the median strand ; all three have split up and rearranged their parts so as to form a single whole. The structure appears to have become endarch as shown in Pl. XIII, Fig. 20, which represents another trace at about the same level.

In the lowest section, 2343, the trace is less prominent, and some more fusion among the various tracheal bands has taken place.

It will be seen that in the behaviour of the trace-bundles after reaching the border of the pith there are considerable differences from *M. Lomaxii* and *M. poroxyloides* ; in the former species the twin bundles fuse immediately on attaining this position ; the centripetal xylem persists far below the point of fusion. In *M. poroxyloides* fusion also takes place high up—almost as soon as the pith is reached. The centripetal xylem of the twin bundles fuses in a definite manner (Scott, 1912, p. 1019), and here also persists for a long way down, after fusion has taken place.

In *M. multirame*, on the other hand, the twin bundles remain distinct for a considerable distance at the edge of the pith. Thus in the case just considered, they are reaching the pith in one section, remain quite distinct through the next two, and only become united into a common mass in the fourth section. There is never in fact a definite *fusion*, such as we see so clearly in the other two species. The two bundles, together with the median strand, merely group themselves in one mass. The centripetal xylem has already ceased to be distinguishable before this grouping is accomplished. The resemblance to *M. Sutcliffii* is much closer (Maslen, 1911, p. 396, Pl. XXXIII, Figs. 3-5) ; in fact, there appears to be no essential difference in the behaviour of the leaf-traces of these two forms.

One or two other points connected with the course of the bundles may be noticed. I have tried to find out whether any regular order is followed in the subdivision (or fusion), according to the direction in which we follow them) of the bundles of the leaf-trace in the cortex. It will be seen in Pl. XI, Fig. 3 that each of the two strands of the double trace is dividing unequally, the outer segment on either side being about twice as broad as the inner. If one follows the trace farther out, one finds, as would be expected, that the outer strand on either side divides again ; this gives two groups of 3 bundles each, with a wide space between them. The next stage shows less constancy ; sometimes the outermost bundle divides once more ; sometimes it is the innermost bundle of the 3 that divides ; the two sides of the same trace may differ in this respect. In any case there are now 8 bundles, which seems to be the regular number entering the base of the leaf. No doubt further subdivisions then go on, as in *M. Sutcliffii*, in which Mr. Maslen observed 16 in a petiole (Maslen, 1911, p. 405). The whole process of subdivision of the trace agrees closely with that in *M. Sutcliffii*, and Mr. Maslen's figures illustrate the essential points sufficiently (l.c., Pl. XXXIV, Figs. 8-10).

Another point is the relation of the axillary stele to the subtending leaf-trace. The stele as it passes in through the cortex and phloem makes an acute angle with the vertical, and consequently is cut almost transversely in transverse sections of the main stem. Immediately on reaching the secondary wood, however, its elements take an almost horizontal course, as shown in Pl. XI, Fig. 3. This is no doubt (like the corresponding curvature of the trace-bundles) an accommodation to the secondary growth of the main axis. As already mentioned, the wood of the axillary stele is connected on either side with the wood on its flanks; sometimes the stele is so much affected by these lateral connexions as to appear split into two lateral halves (cf. Maslen, 1911, p. 407).

Another interesting point is the relation of the axillary stele to the primary bundles of the main stem. This is difficult to make out in a stem which has already undergone a good deal of secondary growth. But, in favourable cases, strands of primary, spiral or scalariform, elements can be seen curving to the right and left at the inner end of the axillary stele, as if to join the adjacent leaf-trace bundles on either side (cf. Pl. XIII, Fig. 14). It appears, then, that the axillary stele in its vascular connexions follows the Coniferous type; it is not directly connected with its own subtending trace, but with the trace-bundles to the right and left, between which its own trace passes out. The same relation was found by Bertrand and Renault in *Poroxylon* (1886, pp. 296, 300-3, Fig. 199), where the insertion of the axillary branch takes place on the two bundles which enclose between them the outgoing leaf-trace.

In tracing the connexions of the bundles I have, for obvious reasons, confined my attention to the xylem.

#### *The Axillary Shoots.*

The axillary shoots are a most characteristic feature of the species and suggested the choice of the specific name *multirame*. As already mentioned, every leaf, through a considerable length of stem, bears a shoot in its axil; in the upper part of the specimen, however, these shoots are undeveloped.

The type-specimen only shows the bases of the axillary shoots. We find these well preserved up to the point where they are just free from the main stem; beyond that, except for some doubtful fragments, they cannot be recognized. The best section for them is 2335, in which 3 are shown, which are nearly or quite free from the stem (see photograph, Pl. XI, Fig. 2). The best preserved is shown in detail in Pl. XIII, Fig. 22. At this level the shoot is absolutely leafless; its outline is more or less elliptical, with the long axis tangential to the parent stem; the dimensions in this case are about 6 x 3 mm. The stele has the remarkably flattened form to which attention has already been called. Its dimensions are about 3.5 mm. long by 0.3 mm. broad. The detached portion at one end might be taken for

a leaf-trace. Similar bodies are shown in two other cases, but it seems certain that their separation from the compressed stele is merely accidental.

The pith is here practically obliterated; in cases where it is rather better preserved 'secretory sacs' with black contents are evident. Centripetal wood has not been recognized, and it is very doubtful if it was present; the centrifugal wood has about five tracheides in each radial row. Where the section is oblique we find that the tracheides are scalariform or spiral, not pitted. The phloem-zone is ill-preserved. The cortex is on the whole of uniform parenchymatous structure, the cells becoming smaller towards the outside; many elements with brown contents, perhaps secretory, are present. In the abaxial half of the shoot, tangential divisions have taken place, both in the inner and middle regions of the cortex. A small-celled epidermis can be traced in many places.

The flattened form of the stele is constant and evidently, in the main, natural, the other tissues showing no sign of compression or distortion. The pith is, however, somewhat more compressed than is natural, as shown by its better preservation in some sections than in others. This excessive compression may be due to growth of the cortical tissues of the shoot, indicated by the frequent occurrence of tangential divisions.

The axillary stele is a good deal better preserved where it has entered the cortex of the stem (Pl. XIII, Fig. 21). Its sectional form is here no longer flattened, but simply elliptical, measuring, in the example figured, about  $1.5 \times 0.75$  mm. This section is cut at the level where the axillary stele has just traversed the cortex and its pericycle is in contact with that of the stem. The pith shows some signs of compression, owing, no doubt, to the secondary growth of the stele in a confined space. Dark (secretory?) elements are present, as before. Groups of thick-walled elements at the inner edge of some of the xylem-wedges seem to represent the centripetal xylem, but I have not been able to determine the position of the protoxylem, so this interpretation is by no means secure. The centrifugal wood has a thickness here of 8–10 elements. It is arranged in definite wedges, the medullary rays between them being broad, owing partly to the size and partly to the number of their cells; though most of the rays appear to be uniseriate, some are three cells wide.

The phloem-zone is well developed—quite half as thick as the wood; in this region the cells of the rays are often tangentially dilated. The zone, with numerous dark-brown elements, beyond the phloem may be regarded as pericycle, but is not well defined.

The axillary stele as it passes through the cortex has essentially the same structure in *M. multirame* as in *M. Sutcliffii* (cf. Pl. XIII, Fig. 21, with Maslen, 1911, Pl. XXXIV, Fig. 11), though on a smaller scale in the latter species. The shoots, however, when they become free are totally different, the little leafy buds of *M. Sutcliffii*, with a cylindrical axis and cylindrical

stele, contrasting strongly with the naked elliptical shoots of *M. multirame* with their remarkable flattened stele (cf. Pl. XIII, Fig. 22, with Maslen's Pl. XXXVI, Fig. 20). Of course this difference cannot be taken as a specific character; it is more likely that the distinction between the two kinds of axillary shoots depends on function; the one, for example, may be vegetative, the other reproductive. There is some additional evidence, given below (p. 452), bearing on the function of the axillary shoots.

#### *The Wood and Phloem.*

The centripetal wood of the leaf-traces is shown in a bundle from the outer cortex (one of eight forming the trace) in Pl. XIII, Fig. 18, and in a double bundle at the margin of the pith in Fig. 19 (see also the photographic Pl. XI, Figs. 3, 4, and 5). In the type-specimen it is perhaps rather less developed than in *M. poroxyloides*. The parenchyma accompanying the protoxylem is present, but in transverse section is not always easily distinguishable from the xylem (Pl. XIII, Fig. 19).

The structure of the secondary wood may be said to be identical with that in *M. poroxyloides*; in particular, we find everywhere a considerable thickness of spiral and scalariform elements (not less than 6–8) before the typical pitted tracheides of the secondary wood begin. In this respect *M. multirame* agrees with *M. poroxyloides* and differs from *M. Sutcliffii* and *M. Lomaxii*, in which the non-pitted type of wood is nearly or quite limited to the neighbourhood of the leaf-traces. The pitted tracheides commonly have two rows of alternating pits on their radial walls—sometimes there is a single row only. The tracheides range from 24 to 36  $\mu$  in diameter; the rays are narrower—about 18–24  $\mu$ .

There is in some parts of the wood a large proportion of very low rays 1–3 cells in height. Higher rays also occur, up to a height of 12–14 cells. Occasionally a ray-cell is found divided radially, making the ray biseriate at that point. The phloem and pericycle show no clear difference from those of *M. poroxyloides*. They are better preserved in another specimen. Immediately outside the pericycle a zone of periderm is already forming (Pl. XI, Fig. 4).

#### *The Cortex.*

The cortex, where completely preserved, is a broad zone 3–3.5 mm. in width. Between the leaf-traces the greater part of this zone is taken up by the mechanical *Dictyoxylon* tissue, which is extremely well developed; only a narrow band of parenchyma intervenes between the pericycle and the beginning of the fibrous strands (Pl. XI, Figs. 1 and 2). But here, as in other species, the outgoing leaf-trace is accompanied by a mass of parenchyma, and at these places the *Dictyoxylon* zone is consequently much reduced in width (Figs. 2 and 3). The fibrous bands of the *Dictyoxylon* cortex often form a network as seen in transverse (Figs. 2 and 3) as well as in tangential

section (Fig. 6). The tangential section shown photographically in Fig. 6, though imperfectly preserved, gives a good idea of the distribution of the leaf-bases, which are recognized at once by the parenchymatous masses traversed by the bundles of the trace; three such leaf-bases are shown; between them the plane of section passes through the *Dictyoxylon* cortex. In connexion with one leaf-base the axillary stele is shown. It will be noticed that the leaf-bases are moderately crowded, but so as to leave a quite appreciable cortical surface between them; here again the structure is much the same as in *M. poroxyloides*.

On the whole there seems to be scarcely any difference between these two species (apart from the probably accidental one of the presence or absence of axillary shoots) except in the mode of fusion of the leaf-trace bundles at the edge of the pith. This, however, is apparently important enough to justify keeping up the distinction.

The affinity to *M. Sutcliffii* is also very close; here the course of the bundles appears to be identical, and the chief distinction is found in the structure of the inner secondary wood, which in *M. multirame* everywhere has numerous layers of scalariform and spiral elements, while in *M. Sutcliffii*, 'with the possible exception of a very few elements on its inner edge, the whole of the secondary xylem-ring, apart from the leaf-traces, consists of pitted tracheides' (Maslen, 1911, p. 399).

#### OTHER SPECIMENS.

A specimen received from Mr. Lomax in 1912<sup>1</sup> requires some special notice as it is in most respects the best-preserved *Mesoxylon* yet discovered, and differs in certain points from the type. Like the latter it is a coal-ball specimen from Shore, Littleborough. A portion about  $1\frac{1}{2}$  inches in length was cut into a series of 13 transverse sections (Nos. 2760–2772) and 8 longitudinal sections (Nos. 2773–2780) were obtained from the rest of the fragment.

It is a relatively large stem, about 4·5 cm. in diameter, if complete. The diameter of the pith is about 19 mm., the thickness of the wood 7–7·5 mm., that of the phloem and pericycle about 3 mm. The cortex is less well preserved than the other tissues, but was apparently about 3 mm. in thickness. The stem was thus a larger one to start with and also more advanced in growth than the type-specimen. The arrangement of the numerous leaf-traces is similar to that already described. As the stem is not quite complete, it was not possible to determine the phyllotaxis exactly; the divergence may have been either  $\frac{8}{21}$  or, more probably,  $\frac{5}{13}$ . The behaviour of the leaf-traces agrees with that in the type; the two bundles of the entering trace remain separate after entering the pith, and there is no

<sup>1</sup> Referred to a previous paper in connexion with *M. poroxyloides* (Scott, 1912, p. 1021).

actual fusion. Axillary steles are present in connexion with some of the leaf-traces.

The points in which this specimen throws further light on the structure of the stem will now be indicated.

While the middle part of the *pith* has perished, only the outer ends of the diaphragms remaining, the persistent external zone is remarkably well preserved. The layers next the wood have somewhat smaller cells than the rest, usually with dark contents, perhaps indicating the presence of starch reserves during life (Pl. XII, Figs. 14, 15). The persistent pith cells generally are isodiametric in transverse section, and appear somewhat flattened (broader than long) when cut longitudinally. There is little elongation even of the peripheral cells. In these respects the specimen differs somewhat from the type (see above, p. 439). The inner layers show evident tangential divisions, suggesting that a medullary periderm was being formed as a barrier towards the central cavity, which, apart from the diaphragms, must have existed during life. The same is the case where the continuity of the persistent zone is interrupted by a radial split. The frequent transverse splits in the persistent zone, however, show no sign of periderm formation, and were evidently due merely to post-mortem shrinkage, which may account in part for the flattened form of the cells.

The details of the course of the *leaf-traces* are shown even better than in the type-specimen. A typical leaf-trace, with its axillary stele, shows the following changes, followed from above downwards.

2761. The trace chosen first appears in the second section, 2761. Here two bundles (one of which is double) are seen somewhat obscurely in oblique section entering the periderm. In the wood opposite this point a double, nearly horizontal, strand is conspicuous, the two parts of the strand 0·4 to 0·45 mm. apart. From evidence adduced below, there is no doubt that this represents the axillary stele of the trace, which is known to have often divided into two in its passage through the wood.

2762. Here the two bundles of the trace, about 1·5 mm. apart, are seen clearly in transverse section in the periderm; one of them is still evidently double. The axillary stele, as such, has disappeared, having already fused with the wood, at this level; but its place is marked by a conspicuous bay in the inner edge of the wood, into which a median strand projects. This is always the indication of an axillary stele having just passed in.

2763. The two bundles of the trace have reached the phloem, and are about 1 mm. apart; at the inner edge of the wood the bay, with the median strand, is very conspicuous.

2764. The trace has passed rapidly inwards and the two strands have now traversed the wood and reached the bay. This quick passage is of course due to the almost horizontal course taken by the trace through the

secondary wood. Having reached the pith the strands turn down again and are cut almost transversely. They are converging somewhat, and between them, separated from each by a medullary ray, is the median strand, now a good deal narrower. The centripetal wood of the leaf-trace strands is well developed, and each is provided with a thick sheath of radially arranged elements, some of which are evidently tracheides. The median strand has no sheath and it is doubtful whether any of its xylem is centripetal, though in other cases it appears to be so.

2765. The two strands are now flush with the inner edge of the wood and are about the width of a strand apart. The median strand is much reduced.

2766. They are somewhat nearer together ; the median strand has disappeared, the two leaf-trace strands are separated by a ray with intercalated narrow radial bands of tracheides. The sheath has practically died out.

2767. The strands are here close together, but still quite distinct, with their centripetal wood still well developed.

2768. Little or no change. At this level the double trace still projects into a bay of the wood.

2769. The double trace is prominent, as before, while the two centripetal groups are still distinct, though reduced ; the centrifugal part of the xylem is no longer in two parts, but consists of six or seven radial bands, one of which is median.

2770. The double trace is still slightly prominent with remains of the two centripetal groups. The centrifugal xylem is scarcely marked off from the rest of the secondary wood.

2771. The trace is no longer recognizable.

It will be seen, on comparing the description of the course of a leaf-trace in the type-specimen, that there is no essential difference. Here also there is never an actual fusion of the two strands ; they merely become merged in the general zone of secondary wood ; the centripetal xylem-groups persist and remain distinct as long as the trace retains its individuality. The sheath, only seen where the twin bundles first reach the pith, is better marked here than in the type. The leaf-traces are very numerous. In one transverse section (Pl. XII, Fig. 15) 13 can be recognized in different parts of their course, and on following the series through the successive sections, many others make their appearance ; the total number found was 28, but possibly they may not quite all represent distinct orthostichies. However, the actual number was undoubtedly greater, as the sections are incomplete. As already mentioned, the phyllotaxis, while not exactly determinable, was evidently a complex spiral, with a most probable divergence of  $\frac{13}{34}$ .

The *axillary steles* are not well represented in the transverse sections ; they are better developed in the part of the stem shown in the longitudinal sections, cut above the transverse series. The best of those in the trans-

verse sections is shown in Pl. XIV, Fig. 23. It is a small strand, only about 0·7 mm. in diameter; the phloem and cambium are well preserved; the wood is very parenchymatous, but tracheides extend to the centre of the stele. It is quite possible that this was a rudimentary or abortive stеле, for those shown in the longitudinal sections are considerably larger, and have a definite pith.

The tangential sections show the changes in the axillary stèle very clearly—one of these stèles can be recognized in the phloem (section 2773); but it is in the wood that the preservation is best. In the outer zone of the wood (section 2774) an axillary stèle (about 1 mm.  $\times$  1·4 mm. in diameter) is cut almost transversely; the pith is elliptical, with the major axis horizontal. The wood of the branch, mostly secondary, is chiefly developed on the lower side, when it passes over directly into the longitudinal strands of the stem-wood: the same is the case laterally, but on the upper side the transition is much more abrupt, the tracheides here running horizontally, often with upward bends. The subtending trace is not visible, though the wood is shown for about 4 mm. below the stèle.

In the next inner section (2775; Pl. XII, Fig. 12) an interesting change appears; the axillary stèle is deeply bilobed, a wedge-shaped incision, filled with stem-wood, extending almost half-way through it from the upper side. The lower and lateral connexions with the stem-wood are essentially the same as before; on the upper side the stem-tracheides converge, to enter the incision. About 4 mm. below the stèle the two strands of the subtending leaf-trace are present, though partly cut away; they are not included in the photograph.

The next section (2776) passes in the middle part, through the persistent zone of the pith, showing the wood, in oblique section, on either side. In the wood, to the left, an axillary stèle (not the same as in the preceding sections) is seen, quite in the inner part of its course (Pl. XII, Fig. 13). The stèle is here completely severed into two, the longitudinal stem-wood extending uninterruptedly between the two halves. The width of the gap, narrowest in the middle, varies from about 0·4 to 0·25 mm. The half-stèles here consist largely of pith. The wood is mostly limited to the upper and outer sides, and is everywhere in continuity with that of the main stem, the lateral connexions being the most extensive.

Thus the occurrence of a division of the axillary stèle into two, where it approaches the primary wood of the stem, is demonstrated. The strand of stem-wood separating the two halves of the divided stèle is no doubt continuous with the median strand observed in the transverse sections.

The division of the axillary stèle is not, however, constant, as is shown by the transverse sections; of five clear cases observed in the series, the stèle was divided in three and undivided in two. One of the latter is represented in Pl. XII, Fig. 14. The stèle abuts on a pith-bay and is clearly

a single strand all through. The tissue in the middle of the stele is pith, and that at its sides wood. There are slight irregularities in the distribution of the tracheides, but no sign of stem-wood interrupting the continuity of the tissues of the axillary stele. The next section below shows a pith-bay and median strand, just as in other cases where a stele has entered.

It thus appears that the division of the axillary stele inwards is an inconstant character of little importance; only its connexions with the stem-wood are essential. Very little is shown in this specimen of the axillary shoots when free from the stem. An example in the first section of the transverse series (2760) has a flattened stele with little secondary wood, and appears to be similar to the shoots so well represented in the type.

One or two points may be mentioned in which this specimen supplements the results obtained from the investigation of the type, as regards *wood and bast*. The pitting on the radial walls of the cells of the rays is fairly shown in places. The number of pits in the 'field' (i. e. in the area where a ray-cell crosses a single tracheide) is from three to six; these pits are elliptical, often oblique, and appear to be simple (Pl. XIV, Fig. 25, m.r.).

A point of interest is the presence of pits on the tangential walls of some of the tracheides, especially as the occurrence of tangential pits in Palaeozoic woods has recently been denied (Jeffrey, 1917, p. 49). They are clearly shown in Pl. XIV, Fig. 24, from a tangential section through the outer part of the wood. Sometimes they form a single row, sometimes two or more; in the latter case they are sometimes very oblique. They are only to be found on a few tracheides, as is shown in transverse sections where the pitting can be distinguished with sufficient clearness to show its distribution.

Tangential pits in *Pitys antiqua* were described and figured in 1902 (Scott, 1902, p. 352, Pl. VI, Fig. 20), but do not seem to have been often met with as yet in wood of Palaeozoic age.

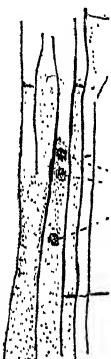
Another feature shown in the specimen under consideration is the presence of wood-parenchyma, which appears at several places. The strand shown in Fig. 25 lies at a distance of about 3 mm. from the pith. It consists of a long vertical row, in part double, of cells with transverse walls. Their length here ranges from about 100 to 200  $\mu$ , with a width of 36-48  $\mu$ . Where the diameter appears about twice as great (84  $\mu$ ) it is possible that longitudinal walls may have broken down. Many of the cells have dark, perhaps resinous contents. Similar rows of cells occur at other places farther out in the wood. Sometimes the individual cells reach a length of about 430  $\mu$ . The cell-walls are rather thinner than those of the tracheides, and appear to be without pits. In some places there is a direct relation to the medullary rays; in one case a vertical strand extends from one ray to another, and appears to cross a third ray on the way. It may be asked whether the parenchyma may not be traumatic; there is apparent evidence of injury in the outer tissues of the stem, for at one place a layer of periderm

cuts deeply into the phloem, enclosing disorganized tissue which had probably died off while the tree was living. There is, however, nothing to show that the wood was affected—it appears quite normal throughout. There are one or two black cracks, filled perhaps with an exudation of resin, but we have no reason to suppose that these lesions occurred during life.

I have observed what appears to be wood-parenchyma in another specimen (section 2578), but the evidence is not so convincing as in the cases just described. The presence of parenchyma in Cordaitean wood is exceptional. I have recorded its occurrence in a remote member of the Cordaitales, *Pitys antiqua* (Scott, 1902, p. 352).

The cambium is preserved in places, but the cells are usually somewhat collapsed. The same applies, to a certain extent, to the inner layers of the *phloem*, but the bulk of this tissue is extremely well preserved.

In transverse section the phloem (about 2 mm. in thickness) is seen to consist of roughly tangential bands of larger and smaller elements (Pl. XI, Figs. 7 and 8). The former are 50–70  $\mu$  in diameter, and are usually almost filled by a brown mass, either solid or with a small central lumen, and somewhat retracted from the cell-wall. In transverse section one might take this mass for thickened cell-wall in an altered condition, but in longitudinal section (Pl. XII, Figs. 10 and 11) it is evident that the brown substance is of the nature of cell-contents; it is irregularly broken



TEXT-FIG. I. From a radial section of the phloem, showing sieve-tubes and parenchyma. *s.p.*, three sieve-plates on radial wall of a sieve-tube.  $\times$  about 105. R.S. S. 2780.

up and resembles the supposed resin-masses often met with in the tracheides or wood-parenchyma. Transverse walls are seldom seen; they may sometimes be hidden by the dark contents, or may have broken down as Bertrand and Renault (1886, p. 292) found in the case of *Poroxylon*.

The smaller elements usually appear clear (Pl. XI, Fig. 8, and Pl. XII, Fig. 11); sometimes they have quite light brown contents, and occasionally they may be infiltrated with the darker substance found in the long tubes. They are often flattened tangentially like cambial cells; the tangential diameter ranges from 50 to 24  $\mu$ , and the radial from 30 to 12  $\mu$ . They have often, as is obvious from the measurements, undergone additional radial divisions.

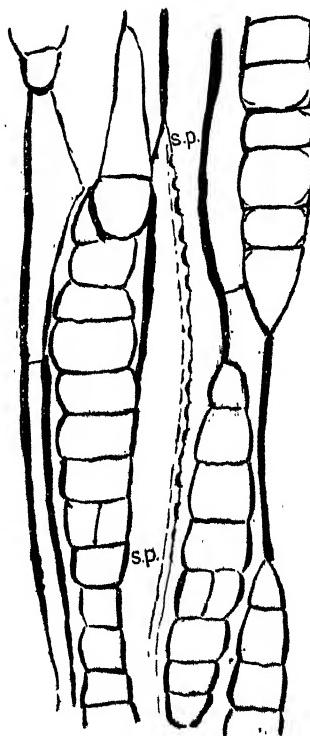
In longitudinal section it is found that these smaller phloem elements are of two kinds; many of them have very distinct and frequent transverse walls; others are long and tapering and not septate (Pl. XII, Fig. 11). The former must be regarded as phloem-parenchyma, the latter as sieve-tubes. It is very rare to find the sieve-plates preserved; but in the case

figured (Text-fig. 1) there are three areas on the radial wall of a tapering element, which show little bright dots on a light-brown background, and appear clearly to be lateral sieve-plates. Further, in a tangential section, the long oblique wall between two such elements shows, in sectional view, a number of thin places marked out in brown, which are no doubt the sections of sieve-plates or fields (Text-fig. 2).

In the outer part of the phloem the parenchyma consists of shorter and wider cells than elsewhere, reaching  $60\ \mu$  in diameter, with a length of  $170-48\ \mu$  (Pl. XII, Figs. 9 and 10). In the same region, elements have been observed with a thick spiral band; whether they were sieve-tubes or not cannot be ascertained. There was evidently some thickening or deposit on the walls, which became drawn out during growth into a spiral form; similar cases have been observed in the phloem of recent Ferns.

We may distinguish, then, three kinds of elements in the phloem—the long, perhaps resiniferous tubes, the sieve-tubes, and the phloem-parenchyma. There remain the phloem-rays. In the outer part of their course they are appreciably dilated—2-3 cells wide, with the cells themselves much enlarged (Pl. XII, Fig. 9). They sometimes have brown contents, like those of the long tubes. Where the phloem abuts on the pericycle, much broader rays are often met with; these are probably principal rays, lying between the groups of primary phloem. The latter are difficult to recognize in transverse section. A slight bulge in the outer margin of the phloem can sometimes be recognized opposite a primary xylem-strand, and occasionally a little group or band of rather thick-walled elements is seen in this position, perhaps representing the altered sieve-tubes of the primary phloem.

The general structure of the phloem of *Mesoxylon multirame* agrees very nearly with that of *M. Sutcliffii*, as described by Mr. Maslen (Maslen, 1911, p. 400). The phloem of *M. Lomaxii* and *M. poroxyloides* is evidently very similar, though the preservation in the observed specimens is not so



TEXT-FIG. 2. From a tangential section of the phloem, showing sieve-tubes, parenchyma, and rays. *s.p.-s.p.*, wall of a sieve-tube, showing numerous sieve-plates in section.  $\times$  about 150. R. S. S. 2773.

favourable as in the present case. On the other hand, the arrangement of the phloem elements in our plant is much less regular than that in *Poroxylon* (Bertrand and Renault, 1886, p. 288), and the supposed resiniferous elements are a much more constant and conspicuous feature.

The pericycle (about 1 mm. in average width, but varying considerably) is distinguished at once from the phloem by the irregular arrangement and larger size of the cells (Pl. XII, Fig. 9). The matrix is formed of short-celled parenchyma, but embedded in this are very numerous and peculiar sacs, of large size, sometimes exceeding  $200\ \mu$  in diameter (Fig. 9), with brown or black contents. In some cases, but not very often, they resemble canals, having a layer or two of flattened cells around the cavity. Often the cavity appears divided into or filled by large cells. The longitudinal sections show that these sacs sometimes consist of a single row of elements, with more or less transverse walls and dark-brown contents. These are no doubt of the same nature as the 'resiniferous' tubes of the phloem. In other cases the sacs, and especially the largest of them, appear irregularly partitioned up into short cells, much exceeding those of the parenchyma in size. It seems that the old sacs have been obliterated, perhaps by tylosis, though no clear evidence of such a process was obtained. The flattened cells surrounding some of the sacs, giving them a canal-like appearance, may be due to a local cambial activity around an effete organ, such as has often been observed in recent plants. The whole structure of these sacs suggests a state of senility.

The *periderm*, formed at the outer border of the pericycle, has reached a thickness of fifteen or more elements. The cells, like those of the pericycle from which they originated, are short. The zone is in places very irregular and may locally form two bands, the inner cutting deeply into the pericycle or even the phloem.

As regards the *cortex*, it need only be said that numerous 'resiniferous' sacs are present in the cellular tissue, and often extend into the bands of sclerenchyma. These sacs sometimes appear septate, but in other cases they are continuous for long distances, perhaps partaking of the nature of the fibres with which they are associated.

From the description of this fine specimen it is evident that the plant must be referred to the species *M. multirame*, for it only differs from the type in quite minor characters. The specimen has been thought worthy of full consideration, as the excellent preservation exhibits in the clearest way the characters of the species and genus.

#### *A Possible Fructification.*

Of the remaining specimens referable to the species *M. multirame*, the only one which requires special notice here is a small stem, 2 cm. in diameter, with the wood about 2 mm. thick.<sup>1</sup> It bore a number of axillary

<sup>1</sup> Sections 2563-2578 in my collection.

shoots, recognizable by their steles, which entirely agree with those of the type. But the particular interest of the specimen lies in the presence of a detached axillary shoot, clearly of the same nature as the others, and itself branched. This shoot is intimately associated with seeds, apparently identical with *Mitrospermum compressum*, A. Arber.

Mr. Lomax has long been convinced that this seed belongs to a *Mesoxylon*, and I am inclined to agree with him, but at present the evidence is not conclusive, for no case of actual connexion between seed and shoot has yet been met with. The subject of the supposed fructifications, of which there are several specimens, must be postponed to another paper, for not all of them appear to belong to the species with which we are now concerned. One point, however, may be emphasized. It is now certain that the axillary shoots of *M. multirame* were branches of a special kind, quite different in their characters from the parent axis. The axillary shoot itself, characterized by its peculiar flattened stele and marked bilateral symmetry, appears to have been naked; there is no clear proof at present that it bore leaves. It branched, however, repeatedly; its branches were distichously arranged, their insertions lying in the principal plane of the narrow stele. The branches bore numerous scale-leaves or bracts, and also other appendages, the nature of which will be discussed elsewhere. The highly specialized character of the axillary branch-systems renders it probable that they were connected with reproduction, and the close association with seeds suggests that they may have constituted the female inflorescences of the plant.

There is evidence from a large specimen, probably belonging to this species, that the stem also branched in an ordinary vegetative manner, the branch in this case repeating the characters of the main axis.

#### SUMMARY.

The summing up of the main characters of the species may take the form of an amended diagnosis:

*Mesoxylon multirame*, Scott and Maslen, 1910.

Leaf-bases moderately crowded, not quite covering the surface of the stem.

Pith large, discoid, with a persistent outer zone.

Twin bundles of the trace remaining distinct for several internodes after reaching the pith, and never definitely fusing before they become merged in the woody zone. Trace dividing into eight bundles in the cortex.

Centripetal xylem persisting about as long as the two strands remain distinct. Sheath variable, limited to the region where the strands first reach the pith.

Tracheides of the whole of the inner part of the wood spiral, reticulate,

or scalariform. Bordered pits in the rest of the wood usually in two rows. Tangential pits present in places.

Medullary rays 1-12 cells in height, usually uniserial. Ray-cells pitted on radial walls. Xylem-parenchyma occasionally present.

Phloem consisting of resiniferous (?) tubes, parenchyma, and sieve-tubes. Phloem-rays dilated.

An axillary shoot present in many of the leaf-axils. Shoot leafless, with a flattened stele, branching distichously, the branches bearing scale-leaves or bracts.

Seam-nodules, Shore, Littleborough. Lower Coal Measures.

It will be noticed that this diagnosis differs in several respects from the one originally given (Scott and Maslen, 1910, p. 238). It has been somewhat expanded as regards the leaf-traces, the wood, the phloem, and the axillary shoots. On the other hand, the cortical characters have been left out, as they seem to afford no real distinction.

The only really important differences from *M. poroxyloides* are in the course of the leaf-traces in the wood and in the axillary steles. The former is a definite and presumably constant distinction; the latter is probably more of biological than taxonomic significance. When we know *M. poroxyloides* better, we shall no doubt find that it sometimes produced some form of axillary shoot, and we have seen that such shoots are not everywhere present in *M. multirame*. As already stated, it is highly probable that the type of axillary shoot characteristic of this species was connected with reproduction.

From *M. Lomaxii* the distinctions are obvious, both in the course of the bundles and the structure of the inner part of the wood.

The latter character also distinguishes *M. multirame* from *M. Sutcliffii*, which further differs widely in the nature of the axillary shoots. This, however, as already pointed out, may be a matter of function rather than of specific distinction.

In the new diagnosis the comparison of the axillary shoot with a phylloclade is omitted, as it might be misleading, now that we know that the shoot represented a branch-system.

The points of more general interest are: The presence of tangential pits on some of the tracheides; the occasional presence of xylem-parenchyma; the structure of the phloem, consisting of more or less concentric bands of secretory sacs (probably resiniferous), sieve-tubes, and parenchyma; the lateral connexions of the axillary stele and its frequent division into two in passing inwards through the wood; the distichous branching of the axillary shoots, the branches bearing scale-leaves or bracts, and also, possibly, secondary branches.

On the whole the characters observed in this species accentuate the relation of the genus *Mesoxylon* to *Cordaites* (Renault, 1879, 1896), but it is

very desirable that more detailed information as to the organization of the stem in the latter genus should be acquired, especially as regards the phloem.

It is hoped soon to publish a full account of the supposed reproductive shoots of the genus *Mesoxylon* and of the anatomy of the isolated species *M. platypodium*.

The photographs in Plates XI and XII are the work of Mr. W. Tams, while the figures in Plates XIII and XIV are from the hands of Mr. G. T. Gwilliam, Miss G. C. Harrison, and in the case of Pl. XIII, Fig. 21, of Mr. J. Allen. The text-figures 1 and 2 were drawn by Mrs. D. H. Scott, F.L.S. To all these collaborators I am much indebted for their indispensable assistance.

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## EXPLANATION OF PLATES XI–XIV.

Illustrating Dr. Scott's paper on the Structure of *Mesoxylon multirame*.

The photographic figures require to be examined with a lens.

## PLATE XI.

Photographs by Mr. W. Tams.

Figs. 1–6. Type.

Fig. 1. General transverse section of stem. An axillary stele is seen on the left.  $\times 3\frac{1}{2}$ . S. 2338.

Fig. 2. Half of a transverse section of stem, showing three axillary shoots, one above (see Fig. 22), one to the right, and one to the left, the two latter still connected with the cortex.  $\times$  slightly over  $3\frac{1}{2}$ . S. 2335.

Fig. 3. Part of transverse section, showing a divided leaf-trace in cortex, the whole thickness of which is shown, as well as the phloem and wood; the axillary stele is passing through the wood.  $\times 13$ . S. 2342.

Fig. 4. Double leaf-trace (No. 14) in pericycle.  $\times$  about 30. S. 2337.

Fig. 5. Transverse section of pith, wood, and phloem, showing a double leaf-trace in a pith-bay. The centripetal xylem of the two strands is clear.  $\times$  about 30. S. 2341.

Fig. 6. Tangential section, passing through three leaf-bases, with the *Dictyoxylon* cortex between them. In each leaf-base the bundles of the trace are seen. a.s. axillary stele, cut obliquely.  $\times 3\frac{1}{2}$ . S. 2344.

Figs. 7, 8. Second Specimen.

Fig. 7. Transverse section of whole thickness of phloem, with part of wood. On the right the large sacs of the pericycle are seen.  $\times 30$ . S. 2769.

Fig. 8. Part of phloem more magnified, showing resiniferous (?) tubes, sieve-tubes, and parenchyma, and phloem-rays, with pericyclic sacs to right.  $\times$  about 75. S. 2769.

## PLATE XII.

Photographs by Mr. W. Tams.

Second Specimen.

Fig. 9. Transverse section of outer part of phloem (to left), pericycle, and part of periderm. The large, more or less obliterated, sacs of the pericycle are evident.  $\times$  about 75. S. 2769.

Fig. 10. Radial section of the whole thickness of the phloem, with part of wood to left. On the extreme right pericyclic sacs are seen.  $\times$  about 30. S. 2778.

Fig. 11. Radial section of part of phloem, more magnified. Here the pericycle is to the left. s. resiniferous (?) sacs; p. phloem-parenchyma; s.t. sieve-tubes; r. phloem-ray.  $\times$  about 75. S. 2778.

Fig. 12. Tangential section through the middle of the wood, showing an axillary stele beginning to divide into two.  $\times$  about 18. S. 2775.

Fig. 13. Approximately tangential section through the inner part of the wood, showing an axillary stele completely divided into two.  $\times$  about 15. S. 2776.

Fig. 14. Transverse section, showing an undivided axillary stele (belonging to trace 12) passing horizontally through the wood, and abutting on a pith-bay.  $\times$  about 25. S. 2760.

Fig. 15. General transverse sections, showing leaf-traces, numbered 1–13, from within outwards (not in order of phyllotaxis, as some are missing). 9, double trace passing through wood; 10, pith-bay with median strand; 11, 12, 13, axillary steles entering through wood. Outside No. 11 the bundles of the subtending trace are seen in the cortex.  $\times$  slightly over 3. S. 2760.

## PLATE XIII.

Drawings. Figs. 16, 17, and 22 by Mr. G. T. Gwilliam; Figs. 18–20 by Miss G. C. Harrison; Fig. 21 by Mr. J. Allen.

## Type.

Fig. 16. Somewhat diagrammatic transverse section of the stem. 3–16, leaf-traces, or (12–16) their axillary steles, numbered from within outwards. In trace 13 the subtending bundles are seen. ?, a doubtful axillary stele. Traces 1 and 2 had already disappeared at this level and trace 15 is lost.  $\times 4$ . S. 2334.

Fig. 17. Radial section.  $c.$  cortex with leaf-trace;  $x.$  wood;  $p.$  discoid pith.  $\times 3$ . S. 2359 (probably from another fragment of the type-specimen).

Fig. 18. Leaf-trace bundle in cortex (one of eight in trace 7).  $px.$  protoxylem;  $x.$  centripetal,  $x_2.$  centrifugal xylem;  $ph.$  phloem.  $\times 165$ . S. 2331.

Fig. 19. Double leaf-trace (No. 12) at border of pith.  $px.$  protoxylem;  $x.$  centripetal,  $x_2.$  centrifugal xylem;  $m.r.$  medullary ray.  $\times$  about 110. S. 2339.

Fig. 20. Leaf-trace cut lower down in its course.  $px.$  protoxylem, here endarch;  $x_2.$  secondary wood;  $m.r.$  medullary ray.  $\times$  about 110. S. 2339.

Fig. 21. Axillary stele in the cortex of stem.  $x.$  centripetal (?) wood;  $x_2.$  secondary wood;  $ph.$  phloem. The lower side is towards the axis.  $\times$  about 60. S. 2339.

Fig. 22. Axillary shoot (No. 16) transverse, with the adjacent cortex of the stem. This is the uppermost shoot shown in Fig. 2.  $d.c.$  *Dictyoxylon* cortex of stem;  $st.$  stele of shoot.  $\times 17$ . S. 2335.

## PLATE XIV.

Drawings by Mr. G. T. Gwilliam.

## Second Specimen.

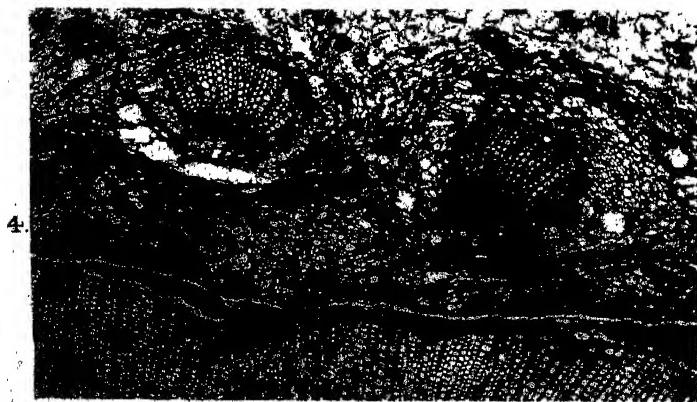
Fig. 23. Small axillary stele in pericycle of stem; the wood extends to the centre.  $ph.$  phloem. The lower side is towards the axis.  $\times$  about 90. S. 2765.

Fig. 24. Tangential section of wood, showing medullary rays and tangential pitting.  $t.p.$  two tracheides with one or more rows of pits on their tangential walls.  $\times 200$ . S. 2774.

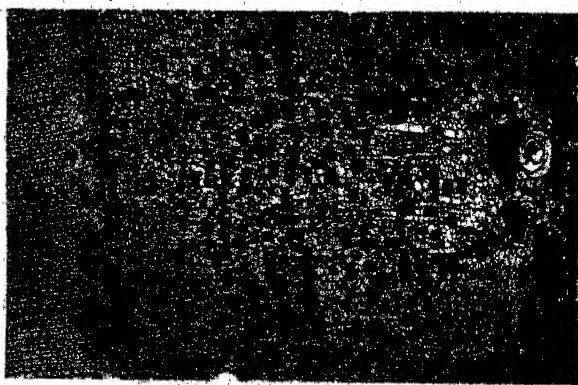
Fig. 25. Radial section of wood, showing pitted tracheides, medullary rays, and xylem-parenchyma.  $x.p.$  xylem-parenchyma, the cells in 1–2 rows.  $m.r.$  medullary ray, showing pitting on the radial cell-walls.  $\times$  about 100. S. 2780.



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7.

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SCOTT-MESOXYLON

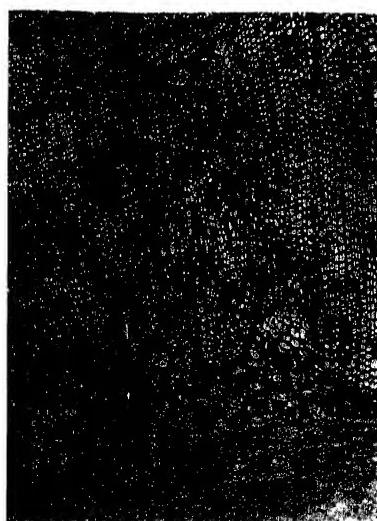
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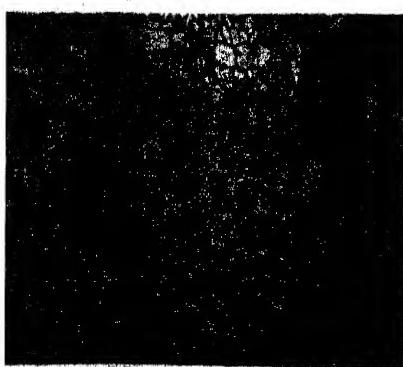
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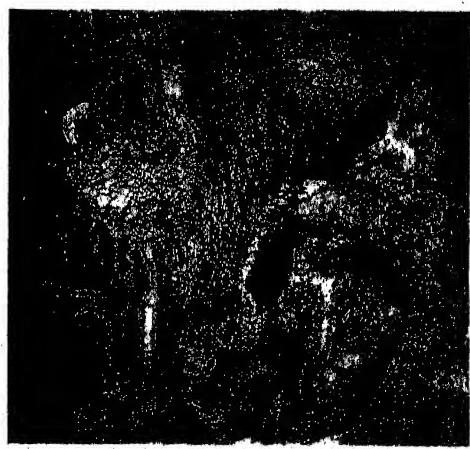
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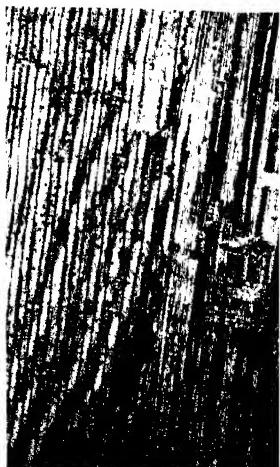


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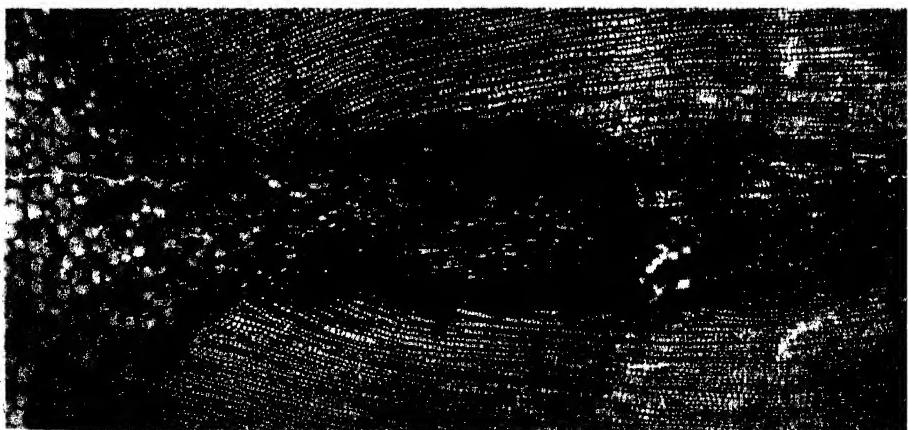




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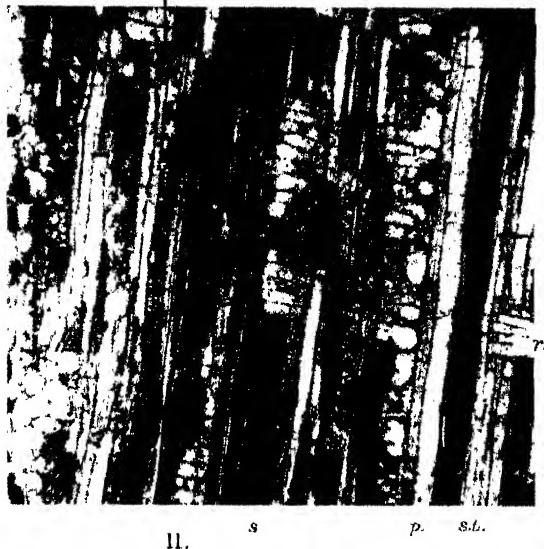
SCOTT—MESOXYLON

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s.t.



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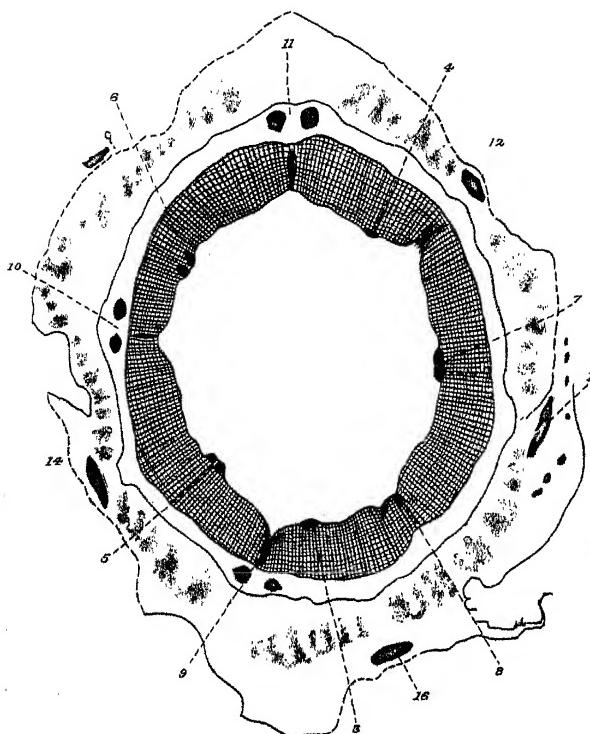


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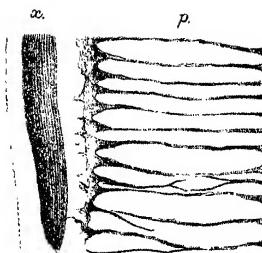


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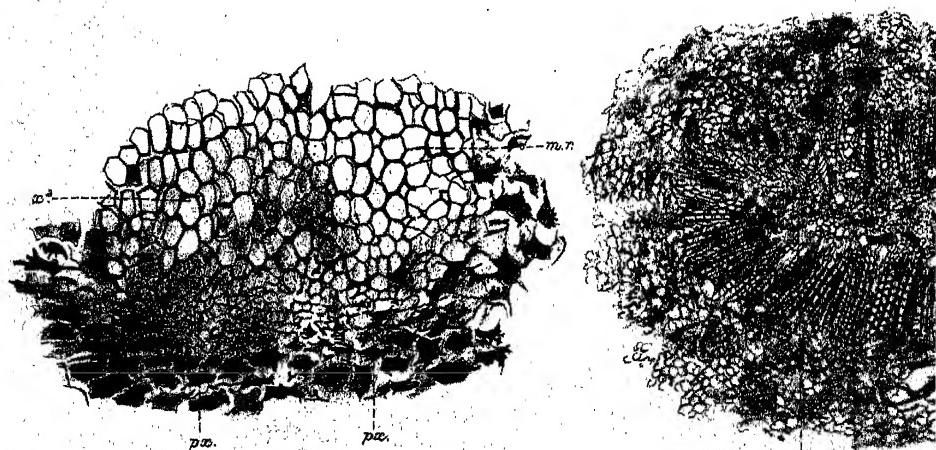
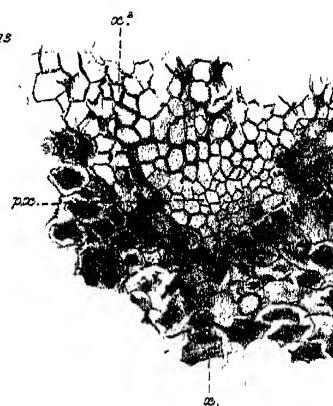
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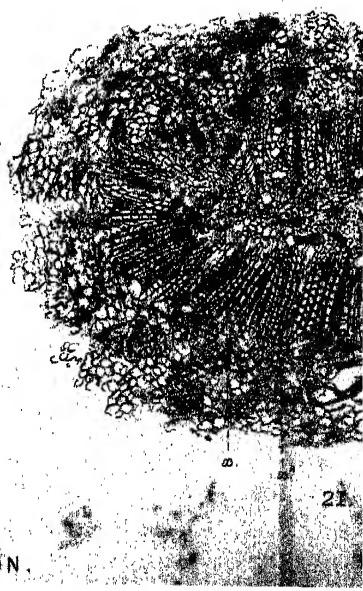
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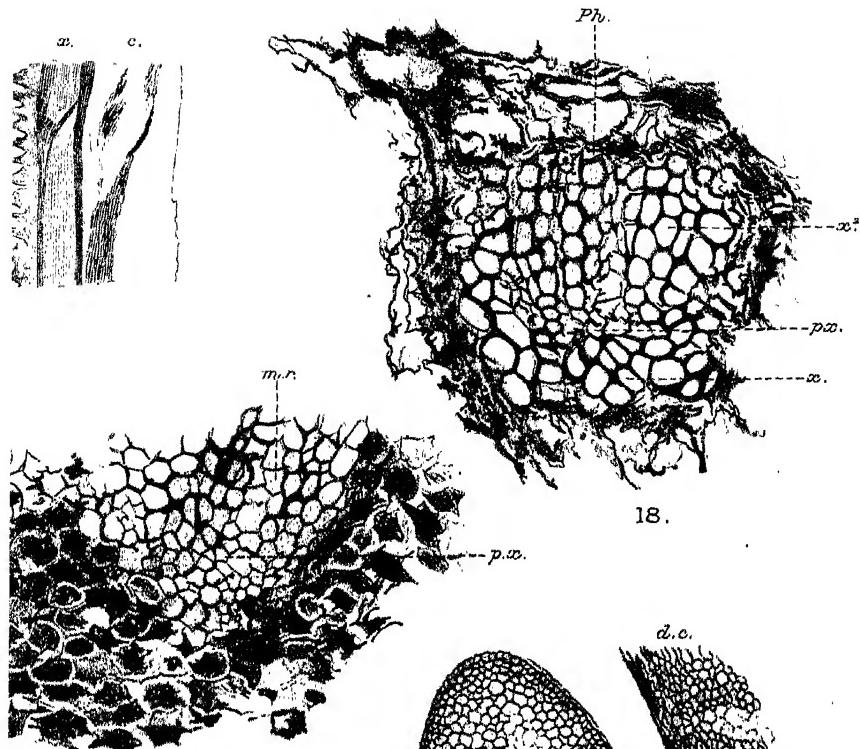
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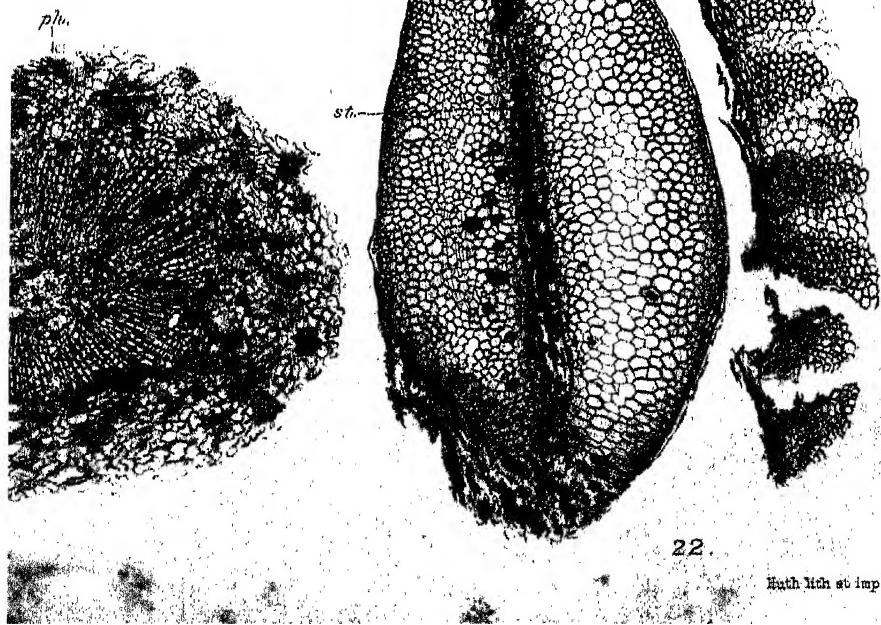
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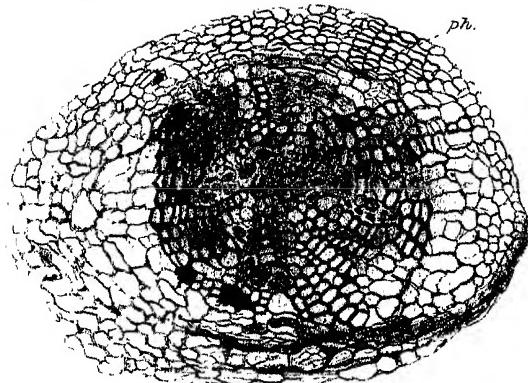
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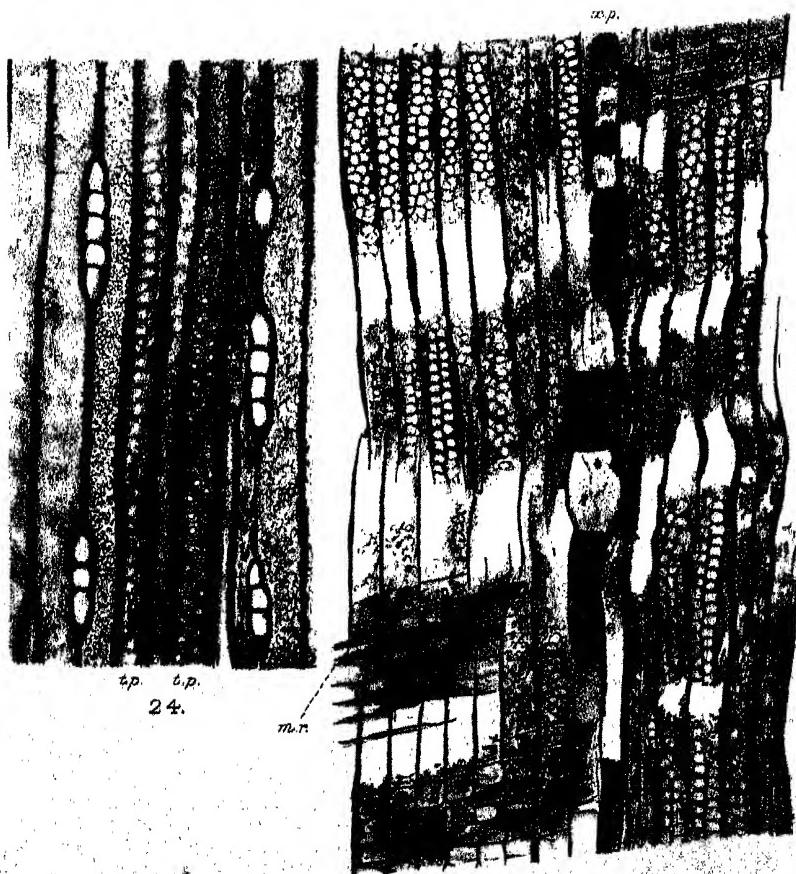
22.

Bath. lith. et imp.





23.



G. William Scott

SCOTT — MESOXYLON



## Cytological Studies in the Protococcales.

### III. Cell Structure and Autospore Formation in *Tetraedron minimum* (A. Br.), Hansg.

BY

GILBERT MORGAN SMITH,

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With Plate XV.

LITTLE is known concerning the cytological structure of members of this genus. Several species are widely distributed in nature, but the alga never occurs in sufficient quantity to fix for cytological study. The observations which I have made upon *Tetraedron minimum* are based upon material obtained by pure culture methods which have been described elsewhere (2). The cultures were grown in 0·2 per cent. Knop's solution and fixed in Flemming's medium osmic-acetic-chromic acid mixture. The fixing solution became diluted a fifth by the addition of the water containing the alga. The washing and dehydration were by means of osmosis through a celloidin film placed over the end of a phial as described in another connexion (3). The material was embedded in paraffin and cut on a microtome in sections  $5\mu$  in thickness. Flemming's triple stain gave the best differentiation of the pyrenoid and nucleus.

The fact that autospores are formed in this genus has been noted by a number of investigators, and the citations compiled by Lagerheim (1), so that no further reference need be made to the subject. Good figures of autospore liberation from the old mother-cell wall are given by West (7). Lagerheim is the only one who has studied the nature of the cell contents and the manner of formation of the autospores. He states that there is a single nucleus and pyrenoid in *T. minimum*. In autospore formation he finds a transverse cleavage dividing the cell contents into two equal parts, and then successive cuttings dividing the cell into a definite number of autospores. Nothing is said concerning the behaviour of the nucleus and pyrenoid during this process, but his figures show a nucleus in each of the daughter-cells.

The youngest cells I have observed always have a single nucleus with a pyrenoid in close association. The nucleus has a very conspicuous

nucleole and nuclear membrane, while the chromatin is indistinct. As the cell grows there is an increase in the size of the nucleus, the chromatin granules becoming more distinct but never standing out sharply in the resting nucleus. In favourable preparations faint linin threads may be seen connecting the chromatin granules.

I have been able to follow the details of nuclear division more fully in this alga than in any other member of the Protococcales that I have studied. The first indication of nuclear division is a decided increase in the size of the nucleus, while certain 'granules' become very prominent. This may be seen by comparing Pl. XV, Figs. 7 and 8, or 22 and 23. The proper interpretation of these 'granules' is a matter of doubt. A fairly definite spireme thread may be seen in the nuclei of certain cells (Fig. 9). These 'granules' may be chromosomes that have been derived by a segmentation of this spireme, or they may be chromatin granules which have become more distinct. In dealing with such small nuclei there is great danger of forcing an interpretation based upon our general knowledge of the process of karyokinesis in other plants; so that it is better to leave the nature and origin of these nuclear 'granules' an open question. In the equatorial plate stage the spindle is distinctly bipolar, the two ends gradually tapering from the mass of chromosomes on the equatorial plate. Little can be said concerning its origin, although the hyaline area surrounding the spindle in Fig. 10 suggests that it is of intranuclear origin. In some preparations there are prominent granules at the poles of the spindle, but no polar radiations (Fig. 33). These polar granules were only noted during the meta-phases. The chromosomes are densely aggregated on the equatorial plate and can only be studied in a polar view of the spindle, when about eight can be seen (Figs. 24, 32). The chromosomes move from the equatorial plate in a dense mass (Fig. 20), and remain bunched after they reach the poles (Fig. 16). Details are lacking concerning the reconstruction of the daughter nuclei.

The cytoplasm of the young cells is densely granular. Occasionally there are vacuoles in growing (Fig. 2), or mature cells (Fig. 8), but this is the exception rather than the rule. Lagerheim (1) has shown that there is a definite parietal chloroplast in *T. minimum*. This stands out quite sharply in living cells, but is difficult to observe in stained preparations. The chloroplast is denser than the rest of the cytoplasm (Fig. 7), which is sometimes very finely reticulated.

Pyrenoids, about the size of the nucleole, are present in the youngest cells. The triple stain is best for differentiating their presence, since the pyrenoid always stains a bright red. I have found that more than one of these organs in the cells of *Characium* (5) and *Pediastrum* (6) is not uncommon, whereas in *Tetradesmus* (3) and *Scenedesmus* (4) it is very rare. In this respect *Tetraedron* resembles *Tetradesmus* and *Scenedesmus*, since not

more than one in ten thousand cells has more than one pyrenoid (Fig. 18). As the cell grows the pyrenoid increases in size (Figs. 1, 11, 14), sometimes becoming irregular in shape (Figs. 3, 4, 5). Perhaps 2 per cent. of the cells show these irregularities. Irregular pyrenoids have been noted in *Characium* and *Pediastrum* and the possibility of their being division stages discussed. If irregular pyrenoids are to be interpreted as division stages, cells with two pyrenoids should be as numerous as those with irregular ones, but since we find that irregular pyrenoids are a hundred times as numerous in *T. minimum* as cells with two pyrenoids, further evidence is furnished that such irregular pyrenoids are not division stages. Irregular pyrenoids can also be seen in living cells, so that they cannot be considered artifacts of fixation. There are also starch plates forming a hollow sphere which encloses the pyrenoid, but these are very delicate and are only noted in the most favourable preparations. In rare instances 'stroma' starch plates, a structure which has been discussed elsewhere (4), were observed (Fig. 13).

There is no definite correlation between the size of the cell and the number of nuclei; since cells of the same size may have one (Fig. 7) or four (Fig. 13). Nuclear divisions are always simultaneous in multinucleate cells and all mitotic figures in such a cell are at the same stage of the process (Figs. 7, 11). Autospore formation may begin in cells containing only two nuclei, or it may not begin until eight nuclei are present, but in the vast majority of cases the process commences in tetranucleate cells. This variation in number of nuclei at the beginning of reproduction has been noted in a number of algae and is connected with the nutrition of the cell, the better nourished ones containing the larger number of nuclei. Stages in autospore formation are more abundant in material fixed during the night, but it is not especially marked at any one time.

The following account is based upon the behaviour of those cells containing four nuclei when autospore formation commences. The first step is a transverse cleavage of the cytoplasm into two equal parts. The line of cleavage is always at right angles to the cell wall and, when the cell is oblong in cross-section, in the shorter diameter. This cleavage is probably very rapid, since no stages were found which showed it in progress. It is similar to that of the other Protococcales which I have studied—*Tetradesmus* (3), *Scenedesmus* (4), *Characium* (5), and *Pediastrum* (6); the pyrenoid remaining unchanged in one of the two daughter-cells (Fig. 21). Since this lack of pyrenoid division has been noted in five members of the order, belonging to four families, it seems a reasonable assumption that it is a generally distributed phenomenon in the Protococcales. It must not be inferred that this generalization is true for all Chlorophyceae, for there are several well-authenticated cases, especially in the Zygnemales, where the pyrenoid divides before cytokinesis.

There is both direct and indirect evidence that karyokinesis follows the

primary cleavage. Fig. 24 shows this nuclear division after the formation of the first cleavage plane. This might be interpreted as a beginning of cleavage before the completion of the usual number of nuclear divisions were it not for the fact that fully 85 per cent. of the cells showing the primary cleavage furrow contained four nuclei, while about the same percentage of cells in the cultures were producing eight autospores. In *Scenedesmus* (4) the single nucleus divides once just before cleavage, and there is a division of the nuclei before each successive series of cytoplasmic cleavages. *Pediastrum* (6), on the other hand, contains a relatively small number of nuclei that divide once or twice just before cleavage, but do not continue dividing after cleavage has started. *Tetraedron*, therefore, combines these two methods, since the cell is multinucleate before cleavage, but the nuclei divide after the first cytoplasmic cleavage.

Two secondary cleavage furrows are now formed at right angles to the primary one. These start at the primary cleavage furrow and are apparently formed by a furrowing in of the plasma membrane (Fig. 26). These furrows run between the nuclei of each daughter-cell, but are not opposite one another in every instance (Fig. 25). Their formation is generally simultaneous, but not always (Figs. 22, 23). The nuclear division which takes place after the completion of the first cleavage may be delayed until these second cleavage furrows have been completed or are well under way. Fig. 26 shows the completion of the second cleavage with the nuclei still in the resting condition, while the nuclei of Fig. 23 have only reached the prophase of division. Since there are eight nuclei at some stage after the completion of the second cleavage (Fig. 27), one further series of cleavages is necessary to make the uninucleate protoplasts. These last cleavage furrows are formed at an angle of forty-five degrees to the plane of the second series (Fig. 29).

After the completion of the first cleavage there is a disappearance of the pyrenoid. Instances were never found where it disappeared before the first cleavage as in *Pediastrum*. The pyrenoid may disappear before the second cleavage (Fig. 22), but as a rule it remains until the completion of the cleavage of the mother-cell into four parts (Fig. 24). Apparently there is a gradual dissolution of this body, its contour changing from a spherical to a lens shape as the process progresses (Fig. 25).

Eight angular protoplasts are found within the old mother-cell wall when cleavage is complete. These are the young autospores. Their shape now changes from the angular to the pillow shape that characterizes the mature cell of *T. minimum*. When a cellulose wall has been secreted the autospores are liberated by a gelatinization or a rupture of the old mother-cell wall. Shortly before this liberation a small pyrenoid is formed *de novo*, the new pyrenoid lying close to the nucleus (Fig. 36).

In *Pediastrum* (6), an alga where the presence of zoospores has been

definitely established, it was noted that at times the zoospores germinated within the old mother-cell wall and appeared like autospores. Search was made for zoospores in *T. minimum* with the possibility in mind that what we have been calling normal autospores are really abnormal germinations of zoospores into vegetative cells within the old mother-cell wall. Very careful observations were made in the hours preceding and following daybreak, because it is at those times that zoospores are generally liberated in the algae. Since they were not found it seems reasonable to assume that the alga reproduces by autospores and not zoospores, but the possibility of zoospore formation in *Tetraedron* is not absolutely excluded, since a normal environment can never be produced in a laboratory culture.

The formation of four, sixteen, and thirty-two autospores by a single cell was also observed. Theoretically the formation of two autospores, or of a multiple of two higher than thirty-two, is possible, but they were not found. In the formation of four autospores the cleavage occurs at the binucleate stage of development (Fig. 19), and then a further cleavage divides the cell contents into four protoplasts. Sixteen autospores are formed when there are eight nuclei in the cell before cleavage. The noteworthy fact in the primary cleavage of such cells is that the number of nuclei in the two daughter-cells is always equal (Fig. 31). In these cells also a nuclear division takes place some time after the formation of the primary cleavage plane (Figs. 32, 33), while the pyrenoid disappears at the same time. The final product of cleavage is a mass of sixteen uninucleate protoplasts (Figs. 34, 35). These are more irregularly arranged than when eight autospores are formed, so that their derivation is harder to follow. The formation of the autospores from these protoplasts takes place as described above. This variation in the number of autospores, as previously stated, rests upon the nutrition of the mother-cell.

#### SUMMARY.

Young cells of *Tetraedron minimum* contain a single nucleus and pyrenoid. Repeated simultaneous karyokineses may produce as many as eight nuclei within a single cell. Autospores are formed by progressive cleavage, the number of nuclei increasing during the process. 4, 8, 16, or 32 uninucleate protoplasts are the final product of this cleavage, these protoplasts being metamorphosed over into autospores. The pyrenoid disappears after the first cleavage, new pyrenoids being formed *de novo* in the young autospores.

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## EXPLANATION OF PLATE XV.

Illustrating Professor Smith's paper on Cell Structure and Autospore Formation in *Tetraedron minimum*.

All the figures were drawn with the aid of the Abbé camera lucida, the drawings being made at the level of the base of the microscope, and with the Leitz oil-immersion objective 1/16 and ocular 4. The magnification is about 2,100 diameters.

Fig. 1. A young cell showing the nucleus with the pyrenoid in close association.

Fig. 2. A slightly older cell.

Figs. 3-5. Cells with irregular pyrenoids.

Fig. 6. A transverse section of a cell.

Fig. 7. A cell showing the chloroplast.

Fig. 8. A prophase of the first nuclear division showing the 'granules' in the nucleus.

Fig. 9. The spireme of the first division.

Fig. 10. Metaphase of the first division.

Fig. 11. Binucleate cells.

Fig. 12. Anaphase of the second nuclear division.

Fig. 13. A cell showing pyrenoid starch and 'stoma' starch.

Figs. 14, 15. Four-nucleate cells.

Fig. 16. Telophases in the third series of nuclear divisions.

Fig. 17. Eight-nucleate cell.

Fig. 18. An abnormal cell with more than one pyrenoid.

Fig. 19. Completion of first cytoplasmic cleavage in the formation of four autospores.

Fig. 20. Anaphases of nuclear division following the first cleavage.

Fig. 21. Completion of first cytoplasmic cleavage in the formation of eight autospores.

Figs. 22, 23. Beginning of second cytoplasmic cleavage.

Fig. 24. Division of nuclei following the first cleavage.

Figs. 25-8. Completion of the second cytoplasmic cleavage.

Fig. 29. The cleavage of the cell into uninucleate protoplasts.

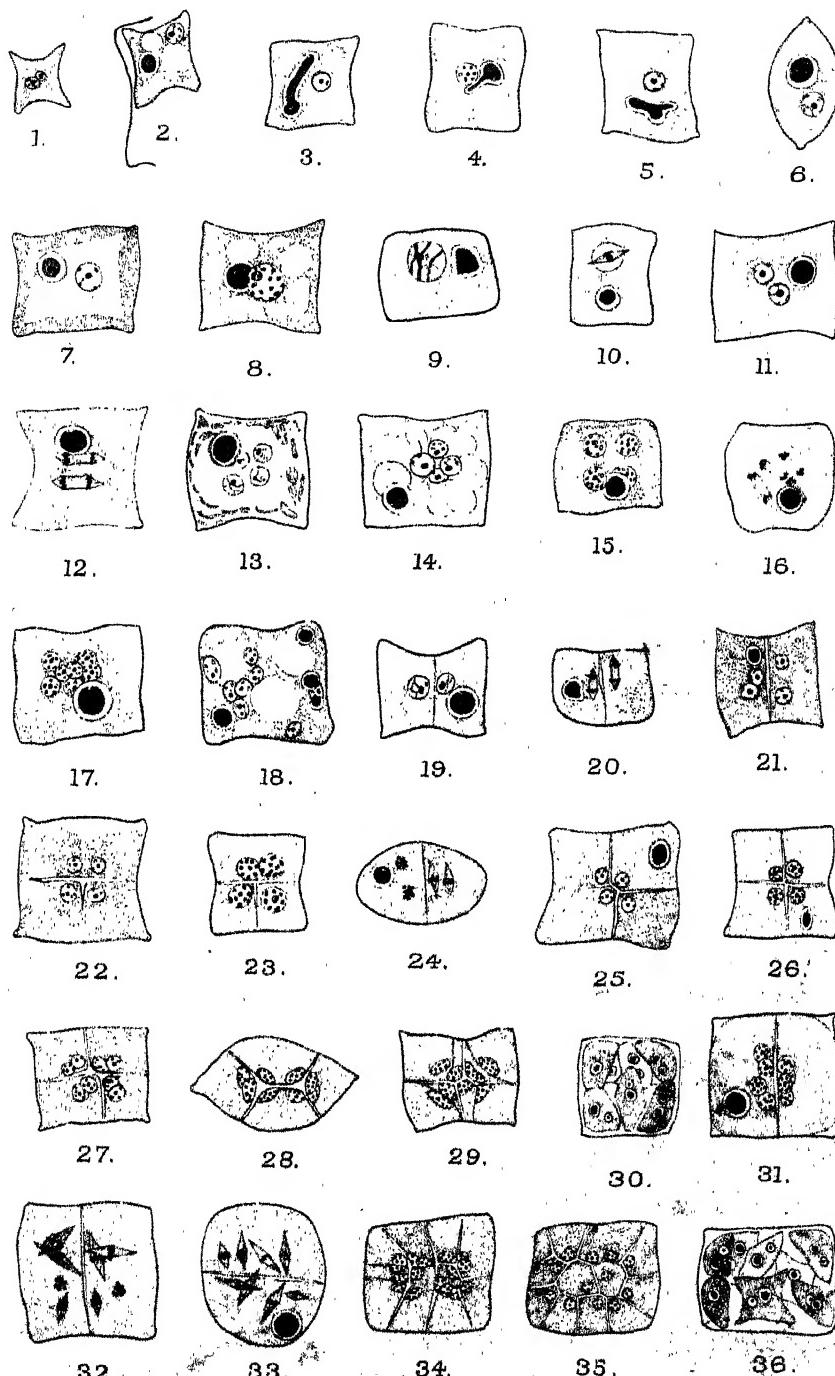
Fig. 30. A later stage in the same, showing young pyrenoids.

Fig. 31. First cleavage in the formation of sixteen autospores.

Figs. 32, 33. The nuclear divisions following the first cleavage.

Figs. 34, 35. Completion of cleavage into sixteen protoplasts.

Fig. 36. Young autospores ready for liberation.





# The Phyllode Theory of the Monocotyledonous Leaf, with Special Reference to Anatomical Evidence.

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With thirty-two Figures in the Text.

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## I. THE PHYLLODE THEORY FROM THE STANDPOINT OF EXTERNAL MORPHOLOGY.

### I. De Candolle's 'Phyllode Theory'.

THERE is now a considerable balance of evidence in favour of the view that the Monocotyledons are descended from Dicotyledonous ancestors. If this theory be accepted, it should become possible to trace homologies between the various organs occurring at the present day in the two groups, since both these groups are thus regarded as the modern representatives of an original common stock. From this point of view, the only structure in the mature plant which presents any difficulty is the leaf. The *typical* Monocotyledonous leaf is of a simple, more or less linear, form, with a sheathing base and parallel veins: how is such a leaf to be compared with that of a Dicotyledon, consisting, in its fullest expression, of leaf-base and stipules, petiole and net-veined lamina?<sup>1</sup> This question has naturally attracted the attention of morphologists, and an interpretation, which has become known as the 'phyllode theory', was put forward, with some reservations, by de Candolle<sup>2</sup> not much less than a century ago. According to this view, the typical Monocotyledonous leaf does not correspond to the complete Dicotyledonous leaf, with its leaf-base and stipules, petiole and lamina, but is merely the equivalent of a *petiole with a sheathing base*. On this interpretation, the Monocotyledonous leaf, in spite of the reduction which it has suffered, still includes within itself, in many cases, parts derived from each of the two developmental regions of the leaf—the 'Oberblatt', which normally produces the lamina and petiole, and the 'Blattgrund' or 'Unterblatt', which gives rise to the leaf-base and stipules;<sup>3</sup> or, to use Bower's<sup>4</sup> terminology, it is derived from the hypodium and mesopodium, the epipodium having been lost. It seems to the present writer probable, however, that in some cases reduction may have gone still farther, so that the leaf-base is alone represented, the leaf thus being derived from the hypopodium only.<sup>5</sup>

The phyllode theory is supported by the existence of a number of examples among Dicotyledons in which organs not dissimilar to typical

<sup>1</sup> In the Phanerogams, with which in this paper we are alone concerned, the differentiation between lamina and petiole has become so firmly established that we are justified in treating these two regions as morphological entities. But the fact that this distinction of parts holds good for the higher plants in no way affects the possibility that the leaf, *as a whole*, may be the modern representative of a thallus-branch, borne by some ancestor of much greater antiquity than the earliest seed plant. See Lignier, O. (1908-9), &c.

<sup>2</sup> Candolle, A. P. de (1827).

<sup>3</sup> Eichler, A. W. (1861).

<sup>4</sup> Bower, F. O. (1884).

<sup>5</sup> In the present paper the term 'phyllode' will be used in a comprehensive sense to include all foliar expansions lacking a lamina, whether they are morphologically equivalent to 'leaf base + petiole' or to 'leaf-base' alone; the two types may be distinguished as 'petiolar phyllode' and 'leaf-base phyllode'.

Monocotyledonous leaves can be shown to be equivalent to leaf-bases, or to both leaf-bases and petioles. Such cases are numerous and familiar, and it is unnecessary here to do more than briefly to recall their existence. Those instances in which the reduced leaves correspond to leaf-bases, with or without stipules, are the commoner. A series of stages can, for example, be traced in such a bud as that of the Black Currant (*Ribes nigrum*, L.), showing transitions from a protective scale with parallel veins to a normal leaf with blade and petiole (Fig. 5 A (i)-(v), p. 474). Similar transitions can be readily followed in the earlier leaves of the long shoots of cultivated Roses and in the expanding bud of the Horse Chestnut. The scale-leaves of *Monotropa*, as de Candolle<sup>1</sup> has pointed out, clearly correspond to the bases of the petioles in the related genus *Pyrola*, while the scale-like prophylls of *Ranunculus Ficaria*, L., are also equivalent to leaf-bases (*pr.* in Fig. 4, p. 474).

The cases in which petiole as well as leaf-base undoubtedly plays a part in the leaf-like expansion are less numerous, but great theoretical interest attaches to them in connexion with the phyllode theory. Certain species of *Oxalis*, e. g. *O. bupleurifolia*, A. St. Hil. (Fig. 3 A, p. 474), show every stage in reduction of the lamina, correlated with a blade-like development of the petiole. The phyllode, in this case, is horizontally expanded, whereas in the numerous phyllodic Acacias it is most commonly flattened in the vertical plane; in one species, however (*A. leptospermoides*, Benth.), the phyllode is described as horizontal,<sup>2</sup> and thus comparable with that of *Oxalis bupleurifolia*, while in others it is not flattened but almost radially symmetrical (e. g. *Acacia scirpifolia*, Meissn., Fig. 1 A and B, p. 474). That the phyllode in this genus is truly petiolar is deduced from the series which can be traced in certain seedlings between normal pinnate leaves with slender petioles—modified leaves with reduced laminæ and flattened petioles—and, finally, phyllodic expansions with no trace of a lamina. Such a series is indicated in Fig. 2 A, p. 474), which represents *Acacia neriifolia*, A. Cunn., but a more complete set of transitional forms can often be found.

It is a commonplace of every text-book that one of the most distinctive features of Monocotyledons is the parallel venation of the leaves; the ‘extraordinary uniformity’ characterizing the main phenomena of venation in the striated type, to which the majority of Monocotyledons belong, was emphasized many years ago by de Bary.<sup>3</sup> But no theory hitherto propounded regarding the origin of Monocotyledons has offered any satisfactory explanation of this well-marked character of the Class. To the present writer it appears that one of the chief merits of de Candolle’s phyllode theory is that it explains the parallel venation of Monocotyledonous leaves in a perfectly unstrained way. For parallel veining is one of the most obvious characters of Dicotyledonous leaf-bases and petioles and of horizon-

<sup>1</sup> Candolle, A. P. de (1827).

<sup>2</sup> Hochreutiner, G. (1896).  
<sup>3</sup> Bary, A. de (1884).

tally expanded phyllodes, so that, on de Candolle's theory, the venation of the Monocotyledonous leaf ceases to present any problem; it shows precisely those characters which might have been anticipated from the morphological nature of the organ. Even the more exceptional types of Monocotyledonous leaves fall into line from this point of view; for among the Iridaceae (p. 485) certain isobilateral, equitant leaves, which are not strictly parallel-veined, reproduce with great exactness the venation of various vertically expanded *Acacia* phyllodes.

## 2. The relation of the 'Phyllode Theory' to the theory of the origin of Monocotyledons through adaptation to the geophilous habit.

In a well-known series of papers, Miss Ethel Sargent<sup>1</sup> set forth the views that Monocotyledons are descended from Dicotyledonous ancestors, and that the single cotyledon of the Monocotyledon is equivalent to the two cotyledons of the Dicotyledon, fused into an apparently single organ. The present writer, who is in entire agreement with these theories, wishes to suggest, as a minor corollary, that the single seed-leaf of the Monocotyledon may, like the foliage leaf, be interpreted as phyllodic; it will then be regarded as representing *the fusion of the leaf-bases and petioles of the two ancestral seed-leaves, the laminae being absent.*<sup>2</sup> The phyllodic interpretation of the Monocotyledonous seed-leaf has the advantage of explaining the frequency, in this Class, of the slender cylindrical cotyledon, showing no differentiation into blade and petiole. A sheathing or winged base is sometimes prominently developed in Monocotyledonous seed-leaves, e. g. *Tigridia*, *Colchicum*, and *Elettaria*,<sup>3</sup> while this region may even be isolated into an apparently distinct organ, as in the coleoptile of the Gramineae.<sup>3</sup> This remarkable development of the cotyledon-base may perhaps be correlated with the loss of a true lamina.

To account for the origin of the cotyledonary fusion in Monocotyledons, Miss Sargent put forward the further theory that this fusion is due to adaptation to the geophilous habit. The present writer would prefer to formulate this view somewhat differently, and to regard the fusion of the two ancestral seed-leaves rather as a structural modification of unknown origin, whose occurrence *facilitated* the adoption of geophilous life, than as an actual *adaptation* to this type of habit. But, with this reservation, she agrees with the essential feature of Miss Sargent's view—namely, that the ancestral Monocotyledon was characteristically geophytic in habit—an idea with which the phyllode theory of the Monocotyledonous leaf is thoroughly in harmony. A study of the leaves of Dicotyledonous geophytes shows that

<sup>1</sup> Sargent, E. (1903), (1904), (1908), &c.

<sup>2</sup> Where a blade occurs it may be regarded as a 'pseudo-lamina' comparable with those of the foliage leaves; see p. 470.

<sup>3</sup> Sargent, E., and Arber, A. (1915).

their petioles play a part of considerable importance. The axis being subterranean, the entire work of raising the leaf lamina into the sunlight devolves upon the leaf-stalk, while its sheathing base often fulfils the additional function of protecting the younger leaves and the flower-buds in their passage through the soil. Salisbury<sup>1</sup> has recently pointed out that, among the plants of our English woodlands, the most specialized type of vernal 'spear shoot', by which geophytes emerge into the upper air, is confined to certain Ranunculaceae and to Monocotyledons. He quotes *Helleborus viridis*, L., and *Ranunculus Ficaria*, L., as showing one or more sheathing scale-leaves (equivalent presumably to leaf-bases) enclosing and protecting the bud;<sup>2</sup> the cells at the apex of the scale are strengthened by thick cell-walls. These examples indicate the value which a reduced leaf may have in the economy of a geophyte. It is probable, in addition, that a system of firm, phyllodic, foliage leaves, with no delicate laminae to injure, might form a bud which would be able to pierce the soil with special ease. There is obviously no *necessary* connexion between geophytism and a phyllodic type of leaf, since there are a vast number of well-established Dicotyledonous geophytes whose leaves have fully-developed laminae; at the same time, a phyllodic leaf might well be a considerable asset to a plant in adopting a geophytic mode of life.

### 3. The relation of the 'Phyllode Theory' to the theory of the aquatic origin of Monocotyledons.

The theory that Monocotyledons owe their peculiar characters to 'self-adaptation to an aquatic habit' has been propounded by Professor Henslow.<sup>3</sup> It is not possible here to enter into the arguments for and against this view: the present writer can only say that it appears to her less probable than the solution proposed by Miss Sargent. But at the same time it may be true that *certain Monocotyledons adopted the water life at a very early period*—in fact, not long after they diverged from the main stock of the Dicotyledons.<sup>4</sup> The Alismataceae, whose flowers possess certain Ranalean features, are probably a case in point. The possible advantage of a narrow, linear leaf to submerged plants, especially to those growing in rapidly-moving water, has been frequently emphasized. It is thus clear that the phyllodic type of leaf is one that can accommodate itself to aquatic life with special ease. This may have been one of the factors concerned in establishing the numerous aquatic Families found among Monocotyledons.

It may well be that the phyllode theory, if accepted, will be claimed by those who adhere to Professor Henslow's view regarding the aquatic origin of Monocotyledons, as affording support to that hypothesis; for the

<sup>1</sup> Salisbury, E. J. (1916).

<sup>2</sup> See Fig. 4, p. 474 of the present paper, for scale-leaves of *Ranunculus Ficaria*.

<sup>3</sup> Henslow, G. (1893 and 1911).

<sup>4</sup> Sargent, E. (1908).

reduction of a normal leaf to a phyllode seems, at first sight, to have taken place in three well-known aquatic Dicotyledons—*Littorella*, *Subularia*, and *Lobelia Dortmanna*, L. But it must be remembered that the phyllode nature of these leaves is not at present proven. To consider their morphological value would occupy space that cannot be spared in the present paper, but the writer hopes, later on, to deal with these leaves, as well as those of certain other Dicotyledons which are of interest from the stand-point of the phyllode theory.

#### 4. The 'Lamina' in certain Monocotyledonous leaves.

So far we have only considered Monocotyledonous leaves in which no lamina is differentiated, but a large number of Monocotyledons existing at the present day possess a distinct lamina, e. g. *Sagittaria*, *Smilax*, various Dioscoreaceae, Araceae, Pontederiaceae, Scitamineae, Palms, &c. How did this lamina arise, and what are its homologies? On the present writer's view of the phyllode theory, the leaf of the ancestral Monocotyledon consisted only of the leaf-base and petiole, and was entirely lacking in lamina. If the Monocotyledons are—as seems most probable—monophyletic, two explanations of the 'lamina' are open to us; it must either be a revival of that organ as it occurs among the Dicotyledons, or an organ which has arisen *de novo*, as a modification of the apical part of the pre-existing phyllode, and thus not strictly homologous with the blade of a Dicotyledon. Professor Henslow,<sup>1</sup> who—without formulating the problem quite in this way—appears to accept the second of these alternatives, has propounded the theory that the so-called lamina of those Monocotyledonous leaves which possess a distinct stalk and blade is merely an expansion of the apical region of the petiole, and thus that the 'aerial reticulated leaf-blades of Monocotyledons are not *identical*, but only *imitative* of the fibro-vascular system of an ordinary dicotyledonous leaf'. This interpretation certainly accords well with the venation of many Monocotyledonous leaves. The arrangement of the veins in *Eichhornia speciosa*, Kunth (Fig. 24, p. 489), for instance, looks decidedly as if the lamina had arisen through a spreading of the apex of the petiole. The transitional leaf forms produced in *Sagittaria* between the band and arrow-shaped types have also all the appearance of merely representing different degrees of expansion of the upper region of the petiole with correspondingly varying degrees of outward curvature and apical detachment of the veins. This series affords an illustration of the way in which the development of the 'pseudo-lamina'—as the present writer proposes to term the leaf-blade of the Monocotyledon—may conceivably have occurred in the course of phyletic history.

One merit of Henslow's theory is that it seems to contain the germ of an explanation of the curious fact that there is a certain general similarity

<sup>1</sup> Henslow, G. (1911).

between the 'laminae' of Monocotyledons belonging to widely separated Families, and also a decided difference between such laminae and those of the Dicotyledons. It is not easy explicitly to define these resemblances and differences, but one or two of the more obvious points may be indicated. The prevalence of forms tending towards the sagittate or cordate—such as those which occur in the Alismataceae (*Sagittaria*), Pontederiaceae (*Monochoria*), Liliaceae (*Smilax*), Dioscoreaceae (*Tamus*), Araceae (*Arum*), and Orchidaceae (*Nervilia*)—becomes less inexplicable if we regard all these laminae as owing their skeletal form to the gradual separation of the originally parallel, petiolar veins. The similarity in the peculiar mode of dissection of the leaves of the Palmae,<sup>1</sup> Cyclanthaceae, and certain Araceae is also a striking feature, since these Families are by no means closely related. Their leaves become more or less deeply incised or actually compound, by a process of necrosis or tearing along certain lines. This method is in no way homologous with the mode of origin of a compound leaf among the Dicotyledons; it suggests that the foliar member exhibiting it may be in an experimental stage of evolution, and that, if we may so express it, it lacks the capacity for forming lobes or pinnae, which is part of the inherited equipment of a Dicotyledon. The tearing into strips, which is constantly suffered by the leaves of the Musaceae, is generally interpreted as an adaptation to a windy climate. But it may, perhaps, rather be regarded as another indication that the 'lamina' of a Monocotyledon is a somewhat imperfect organ, which only succeeds by cumbrous means in approximating to those complex forms which are reached in the Dicotyledons by direct and economical paths.

The greatest difficulty in the way of Henslow's extension of the phyllode theory seems to be that there is very great similarity between the leaf-blades of certain Ranunculaceae and of some of the Alismataceae, although these organs must be supposed, on this theory, to have had a different origin. De Candolle<sup>2</sup> suggested that the leaves of such a plant as *Ranunculus gramineus*, L., might be interpreted as phyllodic, but this view is scarcely borne out by a comparative study of the Family. The nervation throughout the Ranunculaceae is on a uniform plan—the simple, Alismataceae-like leaves being connected by a series of intermediate forms with the more typical cases, which show no obvious resemblance to Monocotyledons.<sup>3</sup> The simpler leaves among the Ranunculaceae may thus be interpreted, not as the more primitive of the types found in the Family, but as reduced forms—an interpretation which minimizes the significance of their resemblance to the 'laminae' of the Alismataceae.

The phyllode theory has met with lively opposition at the hands of Goebel.<sup>4</sup> He discusses the question chiefly in connexion with *Sagittaria*,

<sup>1</sup> Trécul, A. (1853).

<sup>2</sup> Candolle, A. P. de (1827).

<sup>3</sup> Bitter, G. (1897).

<sup>4</sup> Goebel, K. (1891-3).

and takes the view that the band-like submerged leaves of this plant are not reduced leaves, in which the lamina has disappeared and the petiole alone survives, but rudimentary leaves, in which no differentiation of blade from petiole has occurred, and which are 'Hemmungsbildungen' (arrested stages) of the mature form of leaf. He supports this view by recalling that, in the ontogeny of the individual arrow-head leaf, stages are passed through corresponding, first, to the band-shaped submerged leaf and, secondly, to the oval floating leaf. It is true that these developmental facts are not easy to reconcile precisely with the phyllode theory as enunciated by de Candolle, but they fall readily into place when considered in the light of Henslow's extension of de Candolle's view. If the blade of *Sagittaria* be merely an expansion and development of the apical region of the petiole, the band-shaped leaf is indeed, as Goebel says, comparable with a complete air-leaf and not merely with its petiole. Where Henslow would part company with Goebel would be in regarding both the simple band-leaf and the highly differentiated air-leaf as homologous with *the petiole alone* of a typical Dicotyledon.

Henslow's corollary to de Candolle's theory is obviously even more difficult to prove or disprove than the main theory itself. As we shall see in a later section of this paper (p. 488), it gains a considerable degree of support from anatomical evidence.

#### 5. An extension of the 'Phyllode Theory' to the leaves of certain Gymnosperms.

Though the Gymnosperms fall beyond the bounds of our present subject, it may be worth while to touch briefly upon the possibility of an application of the phyllode theory to their case.<sup>1</sup> It seems to the present writer an indication of the validity of this theory, that it affords a point of view which reveals fresh interpretations of leaf morphology in other groups of Seed Plants.

Long ago Asa Gray<sup>2</sup> suggested, as a possible alternative to other explanations, that the leaves of Pines, Cypresses, &c., might be interpreted as homologous with petioles. He, apparently, did not pursue the matter, but his idea seems to the present writer a fertile one. There are many cases among the Gymnosperms to which such an explanation may well be applicable. The uniformity of leaf-structure in the Coniferae is very remarkable. Compton<sup>3</sup> has recently drawn attention to the close relation to one another of the predominant types within the group, and to the association of the narrow acicular, linear-lanceolate, or cypressoid leaf-forms, with the absence of lateral pinnation of the foliar vascular system. He remarks that 'emphasis should be laid on the small power of the Conifers to vary the

<sup>1</sup> The writer hopes to deal more fully with this subject in a later paper.

<sup>2</sup> Gray, A. (1887).

<sup>3</sup> Compton, R. H. (1911).

character of their leaves'. The stereotyped external form and the lack of lateral veins receive a ready explanation if the leaves of the Coniferae are interpreted as phyllodes, equivalent in some cases to petiole and leaf-base, and in others, possibly, to the leaf-base alone. If this view be accepted, the leaves of *Cordaites* and the Coniferae will be regarded as bearing the same relation to those of the Pteridosperms and Cycadophyta as the leaves of Monocotyledons bear to those of Dicotyledons. It is impossible here to enter upon a detailed discussion of the subject, but it may be suggested that the Gnetales offer a special case which is of interest from this standpoint. The leaf of *Gnetum* may be compared with that of a Dicotyledon, while the scale-leaf of *Ephedra* and the parallel veined leaf of *Welwitschia*, with its continued basal growth, would be regarded as petiolar phyllodes—or possibly as leaf-bases only—and thus morphologically comparable with the leaves of Monocotyledons.

The chief general result of the application of the phyllode theory to the Gymnosperms, is that the Coniferae come to be regarded as *microphyllous by reduction*, unlike the Lycopodiales, whose microphyll is probably a primary character of the group.

## II. THE BEARING OF ANATOMICAL EVIDENCE UPON THE PHYLLODE THEORY.

### I. Introduction.

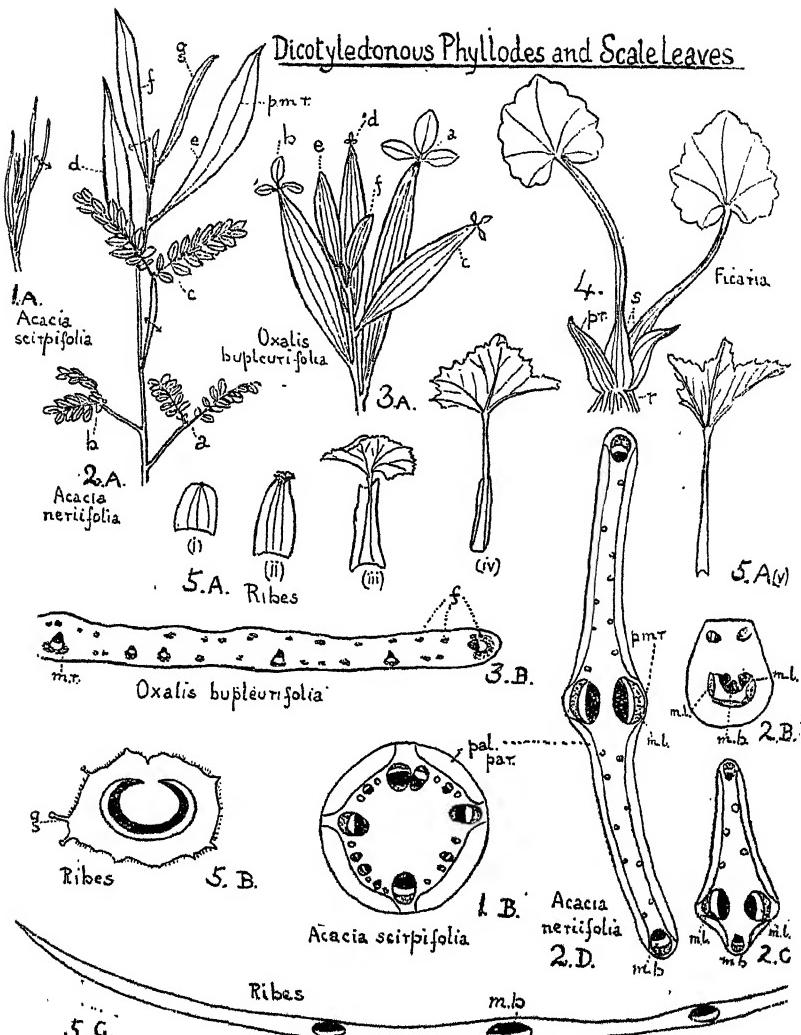
#### (i) *The nature of the evidence.*

The evidence hitherto brought forward for regarding the Monocotyledonous leaf as of a phyllode nature has, apparently, been based entirely upon external morphology. For some years the present writer unsuccessfully pondered the question whether it might not be possible to bring anatomical data to bear on the problem, but she was completely baffled until Solereder,<sup>1</sup> in 1913, reported the discovery of *vascular bundles of inverted orientation* in the leaves of various Hydrocharitaceae. He compared the structure thus revealed to that of petioles, *Acacia* phyllodes, and various isobilateral equitant leaves, but he did not, apparently, attach any theoretical importance to it, or regard it as an indication of the true morphological nature of the leaves of this Family. His results, however, gave the clue the present writer was seeking, and in their light she returned afresh to the problem of how anatomical evidence could be used to test the phyllode theory of the Monocotyledonous leaf.

#### (ii) *The anatomy of Dicotyledonous petioles, phyllodes, and scale-leaves.*

As a preliminary to a general survey of the leaves of Monocotyledons it is necessary to refer very briefly to the anatomical characters of Dicotyle-

<sup>1</sup> Solereder, H. (1913).



Figs. 1-5. (In all diagrams of sections, xylem is represented black, phloem white, and fibres, f, dotted.) Fig. 1 A and B. *Acacia scirpifolia*, Meissn. Fig. 1 A. Apical region of shoot with cylindrical phyllodes (reduced). Fig. 1 B. Transverse section of phyllode ( $\times 30$ ); pal. par. = palisade parenchyma. Fig. 2 A, B, C, D. *Acacia nerifolia*, A. Cunn. Fig. 2 A. Upper part of seedling (reduced); a and b = normal leaves; c = leaf with petiole slightly expanded; d-g = phyllodes; p.m.r. = pseudo-midrib. Fig. 2 B. Transverse section of petiole of a leaf lower on the axis than leaf a. Fig. 2 C. Transverse section of petiole of leaf c; m.b. = median bundle; m.l. = main laterals. Fig. 2 D. Transverse section of phyllode f; p.m.r. = pseudo-midrib, derived from the two main laterals (m.l.); m.b. = median bundle. Figs. 2 B-2 D  $\times 30$ . Fig. 3 A and B. *Oxalis buplexifolia*, A. St. Hil. Fig. 3 A. Apical region of shoot (reduced). The successive leaves, a-d, show progressive reduction of lamina, which in e and f is entirely lost. Fig. 3 B. Transverse section of part of phyllode including midrib, m.r. ( $\times 30$ ). Fig. 4. *Ranunculus Ficaria*, L. Part of basal region of plant to show two prophylls (pr.) corresponding to sheathing bases (s) of normal leaves. Fig. 5 A, B, C. *Ribes nigrum*, L. Fig. 5 A (i)-(v). Successive leaves of bud, showing transitions from bud-scale to normal leaf (reduced). Fig. 5 B. Transverse section of petiole; g = glandular emergence ( $\times 17$ ). Fig. 5 C. Transverse section of bud-scale (incomplete); m.b. = median bundle ( $\times 17$ ).

donous petioles—both those of normal form and those known as ‘ phyllodes’, which are expanded and lamina-like; we must also touch upon the anatomy of those scale-leaves which are equivalent to leaf-bases only.

The structure of petioles<sup>1</sup> varies too much to lend itself readily to generalization. Disregarding a large number of minor complications, we may say, however, that most petioles, as seen in transverse section, are characterized by a more or less open arc or a complete circle of bundles; the result is that, although the majority of the bundles are usually orientated as in the lamina, certain of them, on the ventral or adaxial side, *may come to be inversely orientated*. The position, hence, is that inverted bundles are *absent* in typical Dicotyledonous laminae (excluding the principal ribs) and are *sometimes present and sometimes absent* in petioles; their *presence* is thus a distinctively petiolar character, but their *absence* is a character common to laminae and to some petioles.

The best-known examples of petiolar phyllodes are those found in a number of species of *Acacia* (Figs. 1 A and B and 2 A–D, p. 474, and Fig. 21, p. 483) and *Oxalis* (Fig. 3 A and B, p. 474).

In the case of *Acacia*, the phyllodes, in the majority of species, are expanded in the vertical plane, but they may also be more or less radial in structure (Fig. 1 A and B, p. 474), while in one species they are described as horizontally expanded.<sup>2</sup> The chief anatomical feature in which they diverge from true laminae is in the occurrence of two series of bundles of opposed orientation (Fig. 2 D, p. 474, and Fig. 21, p. 483). Owing to the fact that in some species the earliest leaves of the seedlings have petioles that are scarcely flattened, we are able to trace the changes in the anatomy of the petiole as it becomes phyllodic (Fig. 2 B–D, p. 474).

The phyllodes of *Oxalis bupleurifolia*, A. St. Hil., which are expanded in the horizontal plane, do not show the two series of bundles characteristic of *Acacia*, but have one series of normal bundles, the marginal ones being horizontally placed (Fig. 3 B, p. 474). As an example of a bud-scale we may take that of *Ribes nigrum*, L. In this plant the normal petiole has an arc of xylem and phloem, almost meeting on the upper side to form a complete circle in transverse section (Fig. 5 B, p. 474). But in the bud-scales, which are obviously of leaf-base nature, the vascular supply is reduced to separate parallel bundles orientated as in an ordinary lamina (Fig. 5 C, p. 474).

The study of normal and phyllodic Dicotyledonous petioles and scale-leaves thus leads us to two conclusions: firstly, that the *presence*, in a Monocotyledonous leaf, of additional adaxial bundles with inverted orientation may well be interpreted as affording support to the phyllode theory; secondly, that the *absence* of such inverted bundles would in no way invalidate the theory. For their presence could scarcely be expected

<sup>1</sup> Petit, L. (1887 and 1889).

<sup>2</sup> Hochreutiner, G. (1896).

if the ancestral petiole, from which the phyllode was derived, happened to have an open arc of bundles; inverted strands are also lacking in the phyllodes of *Oxalis bupleurifolia*, whose petiolar nature is uncontested. Again, though some Monocotyledonous leaves are equivalent, on the phyllode theory, to *leaf-bases plus petioles*, it is probable that others are reduced to *leaf-bases alone*, and in these only normally orientated bundles would naturally be found (cf. bud-scales of *Ribes nigrum*). The leaves of certain species of *Iris*, for instance, are best interpreted as corresponding to the leaf-bases alone of other members of the genus.<sup>1</sup>

### (iii) Inverted bundles in Monocotyledonous leaves.

To avoid obscurity, it may be well at this point to anticipate the succeeding sections of this paper so far as to state that the result of a general examination of the leaves of Monocotyledons is to reveal the frequent occurrence of phyllodic anatomy, especially in the more primitive Families. In order to leave no doubt as to what is here intended by the term 'phyllodic anatomy', it may be pointed out that all the cases represented in Figs. 6 to 14, p. 479, show the inverted bundles (*i.b.*) which the present writer regards as indicative of phyllodic structure. Figs. 9 and 10 are instances in which relatively little modification of the original petiolar anatomy has apparently occurred.

### (iv) Midrib anatomy.

It may be objected, with some force, that the type of anatomical structure here called phyllodic might equally well be taken to indicate that the organ showing it is derived from the midrib region of a Dicotyledonous leaf, since many midribs (and some main laterals) closely resemble petioles internally.

On the other hand, there seems to be no positive evidence for the view that the typical Monocotyledonous leaf represents the midrib of an ancestral lamina. The simplicity of form in such a leaf; the uniformity of structure from the top of the leaf-base to the leaf-apex; the lack of any indication of external lateral appendages; the absence of any vestigial internal trace of pinnate or palmate venation;—all these are points suggesting derivation from a leaf-base and petiole alone, rather than a more complex origin.

But since the midribs and main laterals of Dicotyledonous leaves may resemble petioles in structure and may show inverted bundles on the adaxial side, no Monocotyledonous genus is included in the list of phyllodic cases (pp. 478–81) on the strength of its showing inverted bundles *in the main ribs alone*. It is only the occurrence of such bundles outside these ribs which

<sup>1</sup> See p. 485.

can logically be used as evidence of phyllodic origin. For instance, *Melocanna bambusoides*, Trin., of the Gramineae, is not included, although it has inverted bundles in the midrib.<sup>1</sup>

(v) *Trécul's Theory.*

So far as has been ascertained, the only botanist who ever drew morphological conclusions from the existence of the type of leaf anatomy, here called 'phyllodic', was Trécul.<sup>2</sup> In 1876 he published a paper dealing with the Amaryllidaceae, and incidentally with the Liliaceae, in which he compared the anatomy of the leaves of various species of *Agave*, *Narcissus*, *Allium*, and *Aloe* to that of inflorescence axes. He drew the conclusion that these leaves were really stem structures. It is obviously impossible to accept this view for many reasons. Even on anatomical grounds the resemblance of these leaves to petioles is far more precise than their resemblance to stems, since they are generally symmetrical only about a single median plane; this is shown, for instance, in the diagram of an *Allium* leaf, Fig. 9, p. 479. But Trécul's theory, though untenable, is of significance, since it shows that, forty years ago, one botanist had realized that the peculiar leaf anatomy of these Monocotyledons demanded a morphological explanation. The over-insistence on teleological interpretations, which was so rife especially in the latter part of the last century, has tended, in many cases, to obscure the morphological standpoint.

**2. The occurrence of phyllodic leaf anatomy among Monocotyledons.**

(i) *Explanation of list of cases (pp. 478-81).*

We now have to make a general inquiry into the occurrence and distribution of inverted foliar bundles among the various Families of Monocotyledons. In the following table (pp. 478-81) the genera in which phyllodic leaf structure is known to occur are enumerated. In the course of the present study, sections have been cut of selected representatives of most of the Families of Monocotyledons accessible to the writer; those cases of phyllode anatomy whose existence is reported here apparently for the first time are initialed (A.A.), while those in which the present writer has merely confirmed the published accounts are marked with an asterisk. Those in which the leaf is differentiated into petiole and 'lamina' and in which the 'lamina' shows phyllodic structure are marked (L); those marked (E) have isobilateral, equitant leaves with two series of bundles of opposed orientation; the remaining genera have leaves either flattened in the normal horizontal plane, or more or less thickened, or radially symmetrical, but not expanded in the vertical plane. It may be well to note in passing that the present writer regards both isobilateral, equitant leaves and these non-vertical leaves

<sup>1</sup> Brandis, D. (1907).

<sup>2</sup> Trécul, A. (1876).

with inverted bundles as representing two comparable phases of phyllodic structure, between which transitions can be traced; to this point we shall return later.<sup>1</sup>

(ii) List of genera in which phyllodic leaf anatomy has been observed among Monocotyledons.<sup>2</sup>

### HELOBIAE.

- |                   |   |
|-------------------|---|
| POTAMOGETONACEAE. | <i>Cymodocea</i> , sub-genus <i>Phycoschoenus</i> . <sup>3</sup>                          |
| SCHEUCHZERIACEAE. | <i>Triglochin</i> <sup>4</sup> * (Fig. 10, p. 479).<br><i>Scheuchzeria</i> . <sup>5</sup> |
| ALISMATACEAE. (L) | <i>Sagittaria</i> (A.A.) (Fig. 11, p. 479, and Figs. 31 and 32, p. 492).                  |
| BUTOMACEAE.       | <i>Butomus</i> . <sup>6</sup>   |
| HYDROCHARITACEAE. | <i>Enalus</i> . <sup>7</sup><br><i>Stratiotes</i> . <sup>8</sup>                          |
| (L)               | <i>Hydrocharis</i> . <sup>8</sup>   |
| (L)               | <i>Limnobium</i> . <sup>8</sup>   |
| (L)               | <i>Hydromystria</i> . <sup>8</sup>  |

### SPATHIFLORAE.

- |          |  |
|----------|--|
| ARACEAE. | (E) <i>Acorus</i> * (Fig. 18 A and B, p. 483). |
|----------|--|

### FARINOSAE.

- |                    |   |
|--------------------|---|
| PONTEDERIACEAE (L) | <i>Eichhornia</i> (A.A.) (Fig. 24, p. 489, and Fig. 25, p. 490).<br><i>Heteranthera</i> (A.A.) (Figs. 29 and 30, p. 490).<br><i>Pontederia</i> (A.A.) (Fig. 23, p. 489, and Figs. 26 and 27, p. 490). |
|--------------------|---|

### LILIIFLORAE.

- |            |  |
|------------|--|
| JUNCACEAE. | <i>Distichia</i> . <sup>9</sup><br><i>Oxychloe</i> . <sup>9</sup><br><i>Juncus</i> <sup>9</sup> * (certain species). |
|------------|--|

### LILIACEAE.

#### Melianthoideae.

##### Tofieldiae.

- |     |  |
|-----|--|
| (E) | <i>Narthecium</i> . <sup>10</sup> *  |
| (E) | <i>Tofieldia</i> <sup>10</sup> * (Fig. 20, A-C, p. 483).<br><i>Pleea</i> . <sup>10</sup> |
| (E) | <i>Nietneria</i> . <sup>10</sup>   |

<sup>1</sup> p. 482.

<sup>2</sup> In this list the Families are arranged mainly according to A. Engler and E. Gilg's Syllabus der Pflanzenfamilien, 7th edition, 1912.

<sup>3</sup> Sauvagean, C. (1891). See note on p. 481. <sup>4</sup> Areschoug, F. W. C. (1878). See note on p. 482.

<sup>5</sup> Raunkær, C. (1896).

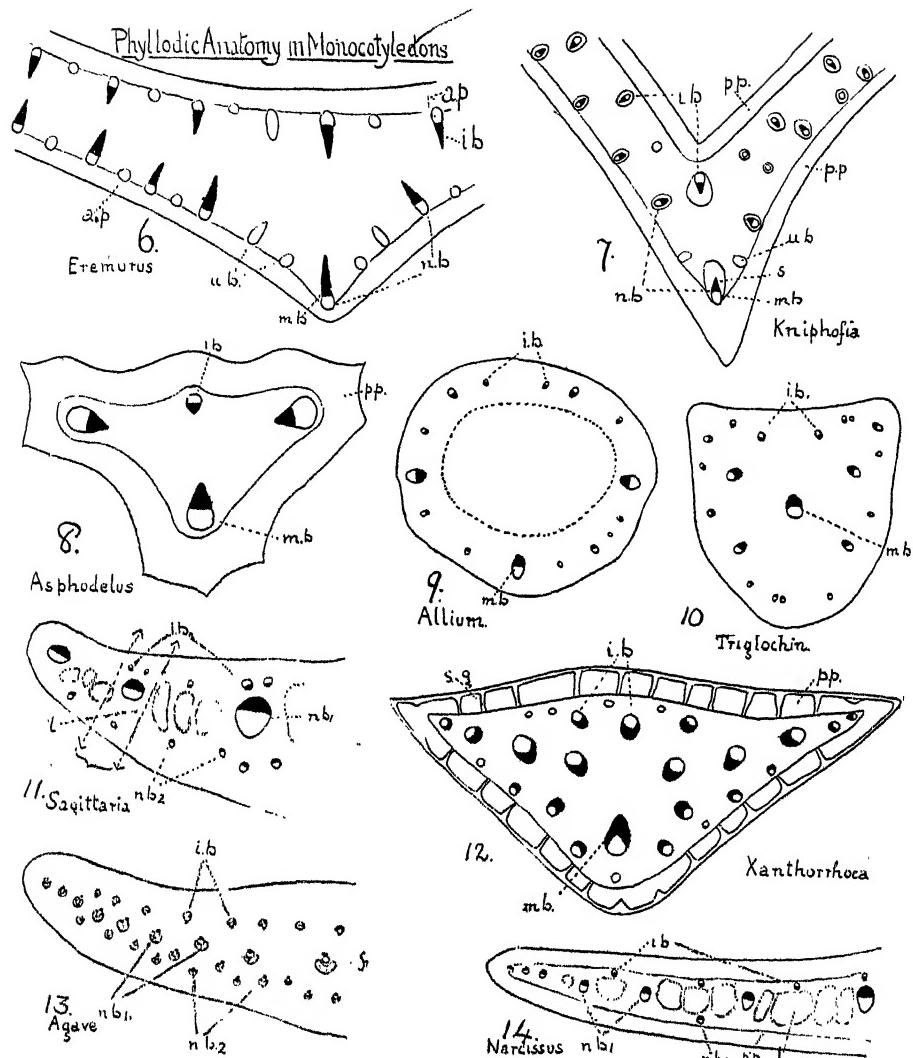
<sup>6</sup> Magnus, P. (1870).

<sup>7</sup> Buchenau, F. (1906).

<sup>8</sup> Sauvagean, C. (1893). See note on p. 482.

<sup>9</sup> Solereder, H. (1913).

<sup>10</sup> Schulze, R. (1893).



FIGS. 6-14. (Lettering throughout as follows:—*m.b.* = median bundle; *n.b.* = normal bundle; *n.b.<sub>1</sub>* = normal bundle of main series; *n.b.<sub>2</sub>* = normal bundle of second series; *i.b.* = inverted bundle; *u.b.* = undifferentiated bundle; *f.* = fibres; *l.* = lacuna; *p.p.* = palisade parenchyma; *ap.* = assimilating parenchyma. Xylem throughout represented solid black, phloem white, and fibres dotted.) Fig. 6. *Eremurus himalaicus*, Baker. Transverse section of midrib and adjacent region of very young leaf ( $\times 14$ ). Fig. 7. *Kniphofia caulescens*, Baker. Transverse section of midrib region of leaf ( $\times 14$ ). Fig. 8. *Asphodelus tenuis*, L. Transverse section of leaf ( $\times 47$ ). Fig. 9. *Allium Cepa*, L. Transverse section of upper part of leaf ( $\times 14$ ). Fig. 10. *Triglochin maritimum*, L. Transverse section of leaf ( $\times 23$ ). Fig. 11. *Sagittaria montevidensis*, Cham and Schlecht. Transverse section of part of leaf, including margin ( $\times 23$ ). The part enclosed between the arrows is shown in detail in Fig. 32, p. 492. Fig. 12. *Xanthorrhoea* sp. Transverse section of leaf ( $\times 14$ ). Hypoderm and girders (*s.g.*) sclerised. Fig. 13. *Agave densiflora*, Hook. Transverse section of part of leaf, including margin ( $\times 14$ ). Fig. 14. *Narcissus pseudo-narcissus*, L. Transverse section of part of leaf, including margin ( $\times 23$ ).

**Asphodeloideae.****Asphodelinae.***Asphodelus*<sup>1</sup>\* (Fig. 8, p. 479).*Eremurus* (A.A.) (Fig. 6, p. 479).**Anthericinae.***Bulbine* (A.A.).**Kniphofinae.***Kniphofia*<sup>2</sup> (Fig. 7, p. 479).*Notosceprium*.<sup>1</sup>**Aloinae.***Aloe*<sup>3</sup>\**Gasteria*<sup>4</sup>\**Harworthia*<sup>4</sup>\**Lomatophyllum*.<sup>4</sup>*Apicra*.<sup>5</sup>**Johnsoniaeae.***Stawellia*.<sup>1</sup>*Johnsonia*.<sup>1</sup>**Lomandreae.***Xanthorrhoea* (A.A.) (Fig. 12, p. 479).*Xerotes* (*Lomandra*).<sup>6</sup>**Allioideae.****Allieae.***Allium*<sup>3</sup>\* (certain species) (Fig. 9, p. 479).*Milla*.<sup>8</sup>*Stropholirion*.<sup>6</sup>*Gagea*.<sup>6</sup>\***HAEMODORACEAE.**(E) *Lachnanthes*.<sup>7</sup>(E) *Haemodorum*.<sup>6</sup>**AMARYLLIDACEAE.****Amaryllidoideae****Amaryllideae.***Amaryllis*.<sup>8</sup>**Narcisseae.***Narcissus*<sup>9</sup>\* (Fig. 14, p. 479).<sup>1</sup> Schulze, R. (1893).<sup>2</sup> Berger, A. (1908).<sup>3</sup> Trécul, A. (1872).<sup>4</sup> Trécul, A. (1872), and Prolius, F. (1884).<sup>5</sup> Prolius, F. (1884).<sup>6</sup> Schmidt, C. (1891).<sup>7</sup> Scharf, W. (1892).<sup>8</sup> Re, L. (1894). The observation relates to *Amaryllis nivea*, Schult.<sup>9</sup> Trécul, A. (1876).

## Agavoideae.

*Agave*<sup>1</sup>\* (Fig. 13, p. 479).

## Hypoxidoideae.

## Conostylideae.

(E) *Conostylis*.<sup>2</sup>(E) *Blancoa*.<sup>2</sup>(E) *Anigozanthos*<sup>2</sup>\* (Fig. 19 A and B, p. 483).(E) *Phlebocarya*.<sup>2</sup>IRIDACEAE.<sup>3</sup>

## Crocoideae.

*Romulea*.<sup>4</sup>

## Iridoideae.

## Iridineae.

(E) *Hermodactylus*.<sup>5</sup>(E) *Iris*\* (certain species) (Fig. 17 A and B, p. 483).(E) *Moraea*.<sup>6</sup>\*

## Sisyrinchieae.

(E) *Diplarrhena*.<sup>5</sup>\*(E) *Libertia*.<sup>5</sup>\**Bobartia*.<sup>4</sup>\**Sisyrinchium*<sup>5</sup>\* (Fig. 16 A and B, p. 483).

## Aristeae.

(E) *Patersonia*.<sup>4</sup>(E) *Aristea*.<sup>7</sup>

## Ixioideae.

## Gladioleae.

(E) *Tritonia*<sup>6</sup>\* (Fig. 15 A-D, p. 483).(E) *Gladiolus*.<sup>5</sup>\*

## ORCHIDACEAE.

(E) *Dendrobium anceps*, Sw. (*Aporum anceps*, Lindl.) (A.A.).(E) *Maxillaria iridifolia*, Reichb. (A.A.).

## 3. Notes upon certain of the non-equitant and non-laminate cases enumerated.

## POTAMOGETONACEAE.

*Cymodocea isoetifolia*, Aschers. Sauvageau<sup>8</sup> describes the upper part of the cylindrical, rush-like leaf as showing a normally orientated median bundle and, in addition, a peripheral series of bundles with the xylem directed inwards.

<sup>1</sup> Trécul, A. (1876).<sup>2</sup> Schmidt, C. (1891).

<sup>3</sup> The cases of phyllodic anatomy in this Family are so numerous that only certain typical genera are cited in this list.

<sup>4</sup> Pax, F. (1888).<sup>5</sup> Chodat, R., and Balicka-Iwanowska, G. (1892). See also Balicka-Iwanowska, G. (1892-3).<sup>6</sup> Species examined, *M. iridioides*, L.<sup>7</sup> Scott, D. H., and Brebner, G. (1893).<sup>8</sup> Sauvageau, C. (1891).

## SCHEUCHZERIACEAE.

*Triglochin maritimum*, L. In the solid, non-sheathing upper part of the leaf, the present writer has found, in agreement with Areschoug's<sup>1</sup> observation, a median, normally orientated bundle, two main laterals obliquely placed, and a series of peripheral bundles with the xylem directed inwards; the peripheral bundles towards the adaxial face are thus inversely orientated as compared with those of a normal lamina (Fig. 10, p. 479). T. G. Hill, who has also described the anatomy of the leaf, figures the vascular strands towards the adaxial surface as though their xylem were directed upwards.<sup>2</sup> This is apparently an error; the bundles are small and their inversion may easily be overlooked.

*Scheuchzeria palustris*, L. According to Raunkiær,<sup>3</sup> the anatomy of the leaf of this plant is essentially similar to that of *Triglochin maritimum*.

## BUTOMACEAE.

*Butomus umbellatus*, L. Sauvageau<sup>4</sup> describes the leaves as phyllodic; he says that the triangular transverse section shows an arc of normal bundles and a peripheral series of numerous small bundles. Sauvageau does not explicitly describe the orientation of these outer bundles, which seem to be somewhat reduced.

JUNCACEAE, LILIACEAE, AND AMARYLLIDACEAE.<sup>5</sup>

It is unnecessary to enter into details here concerning these Families, since it has long been known that they contain, besides numerous cases of leaves with a single row of normally orientated bundles, other cases of leaves thickened in various degrees, or even almost radially symmetrical, in which inverted bundles with the xylem directed downwards occur towards the adaxial face (Figs. 6–9, 12–14, p. 479). Such a leaf as that of *Allium Cepa*, L. (Fig. 9, p. 479), may be closely compared with a cylindrical Dicotyledonous phyllode, such as that of *Acacia scirpifolia*, Meissn. (Fig. 1 B, p. 474). A third class of cases is even more striking from the point of view of the phyllode theory—that, namely, in which the leaf is not markedly thick, but in which there is, nevertheless, a series of inverted bundles towards the adaxial face, in addition to the normal series, e.g. *Eremurus himalaicus*, Baker (Fig. 6, p. 479), and *Narcissus pseudo-narcissus*, L. (Fig. 14, p. 479). The isobilateral equitant leaves belonging to these and other Families we shall consider in the next section of this paper.

#### 4. The isobilateral equitant leaf and its relation to other phyllodic types.

The type of leaf occurring in many species of *Iris* and described as 'equitant' is, as is well known, characterized by a sheathing leaf-base,

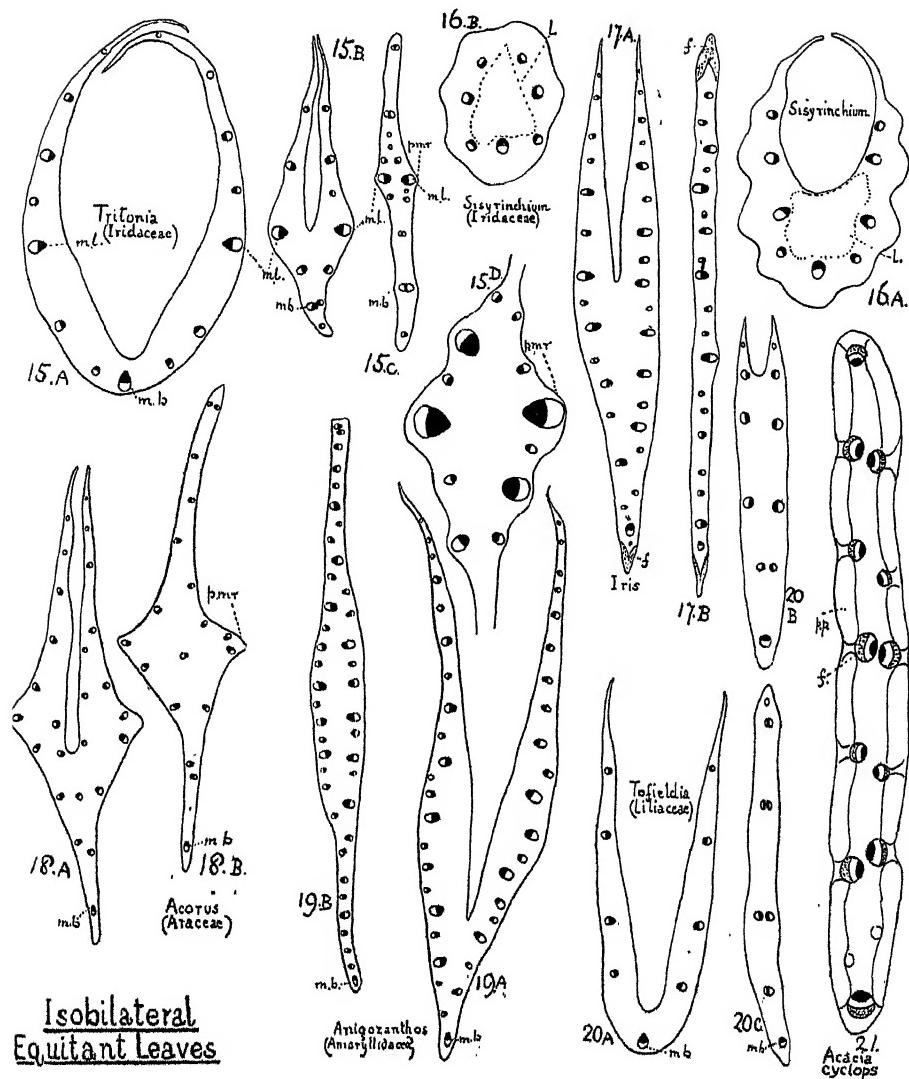
<sup>1</sup> Areschoug, F. W. C. (1878).

<sup>2</sup> Hill, T. G. (1900), Pl. VI, Fig. 7.

<sup>3</sup> Raunkiær, C. (1896).

<sup>4</sup> Sauvageau, C. (1893).

<sup>5</sup> The present writer proposes to deal further with these Families in a later paper.



Figs. 15-21. (Lettering throughout :—m.b. = median bundle; m.l. = main lateral; l. = lacuna; f. = fibres; p.m.r. = palisade parenchyma. Xylem is represented solid black, phloem white, and fibres dotted. Fig. 15 A-D. *Tritonia* (garden hybrid). Fig. 15 A, B, C. Successive transverse sections of young leaf, passing through basal sheathing, and upper regions. Fig. 15 D. The pseudo-midrib (p.m.r.) from an older leaf ( $\times 23$ ). Fig. 16 A and B. Transverse sections of sheathing and upper regions of leaf of *Sisyrinchium* sp. ( $\times 23$  circa). Fig. 17 A and B. *Iris* sp. Transverse sections of sheathing and upper regions of leaf ( $\times 14$ ). Fig. 18 A and B. *Acorus Calamus*, L. Transverse sections of sheathing and upper regions of leaf ( $\times 7$ ). p.m.r. = pseudo-midrib. A series of very small bundles lying close to both surfaces between the larger bundles has, for simplicity, been omitted. Fig. 19 A and B. *Anigozanthos* sp. Transverse sections of sheathing and upper regions of leaf ( $\times 7$ ). Fig. 20 A-C. *Tofieldia calyculata*, Wahl. Series of transverse sections through basal sheathing and upper regions of leaf ( $\times 23$ ). Fibrous bundle-sheaths not represented. Fig. 21. Transverse section of phyllode of *Acacia cyclops*, A. Cunn. ( $\times 23$ ). Some of the smaller bundles omitted.

succeeded by an upper region flattened in the vertical plane and isobilateral in its anatomy ; the phyllotaxy is distichous. The appearance of such a leaf at first glance suggests the idea that it has been folded along the midrib and that the two halves of the upper surface have fused ; indeed, the opinion that the peculiarities of this type of leaf are due to congenital fusion is, apparently, widely held.<sup>1</sup> The actual evidence for this view is, however, somewhat slender : neither ontogeny nor comparative morphology afford it much support. The leaf, according to Goebel,<sup>2</sup> develops by means of two growing points, one belonging to the sheath and the other to the ' lamina '. The ' lamina ', except where it passes into the sheath, is solid from the first. Goebel shows that the development of the leaf is similar in all essentials to that of the ' radial ' leaves of *Juncus* and *Allium*, and he concludes that there is, in the case of *Iris*, no ontogenetic evidence for the concrescence of two surfaces. Fifty years previously, a similar conclusion had been reached by Trécul,<sup>3</sup> who wrote : ' Il n'y a point ici de soudure ; la feuille naît telle que nous la connaissons. '

It seems to the present writer that the chief obstacle to a rational interpretation of the *Iris* leaf has been the fact that it is too often considered as a case apart and unparalleled. This notion has been fostered by its treatment in many elementary botanical classes as if it were a unique type. But isobilateral equitant leaves occur in the Iridaceae, and also in the Araceae, Liliaceae, Haemodoraceae, Amaryllidaceae, Restiaceae,<sup>4</sup> Philydraceae, Xyridaceae, and Orchidaceae. It is also perhaps significant that the three central Families, Liliaceae, Iridaceae, and Amaryllidaceae, contain, in addition to genera with isobilateral equitant leaves, other genera with non-equitant leaves, showing the type of structure here called phyllodic. The present writer wishes to put forward, as an alternative to the ' congenital concrescence ' theory, the view that *the isobilateral equitant leaf is merely a phyllode flattened in the vertical plane, and thus comparable with the great majority of Acacia phyllodes.*

The best justification for this view—which brings the isobilateral equitant leaf into close relation with the other Monocotyledonous leaves exhibiting phyllodic anatomy—is to be found in a comparative study of the Iridaceae.

Among the Iridaceae there are, besides the vertical equitant leaves, others, such as that of *Sisyrinchium* (Fig. 16 A and B, p. 483), in which the upper region is almost radially symmetrical. Such a leaf is closely comparable with the phyllodic leaf of *Allium Cepa* (Fig. 9, p. 479), and with the phyllodes of *Acacia scirpifolia* (Fig. 1 B, p. 474) and of *Rhyticarpus difformis*.<sup>5</sup> We need only imagine the leaf of *Sisyrinchium* flattened and

<sup>1</sup> Chodat, R., and Balicka-Iwanowska, G. (1892); Massart, G. (1894).

<sup>2</sup> Goebel, K. (1905).

<sup>4</sup> *Anarthria*, according to Brown, R. (1810).

<sup>3</sup> Trécul, A. (1853).

<sup>5</sup> Briquet, J. (1897).

expanded in the vertical plane to produce an isobilateral leaf similar to the equitant leaf of *Tofieldia* (Liliaceae, Fig. 20 A-C, p. 483) or *Iris* (Fig. 17 A and B, p. 483). This comparison is facilitated by the fact that some *Iris* leaves, e. g. those of *I. tenuifolia*,<sup>1</sup> have an oval transverse section, forming, as it were, a transition towards the *Sisyrinchium* type. That vertical flattening is in itself of no morphological significance, is demonstrated by the comparison of the cylindrical and flattened petioles occurring in different species, or even in different ontogenetic phases of the same species, within the genus *Acacia*.

Certain species within the genus *Iris*, such as *I. persica*,<sup>2</sup> L., and *I. orchoides*, Carr., have leaves expanded in the horizontal plane with no flattened vertical region. These leaves are possibly equivalent to the leaf-bases alone in the case of the other Irises.<sup>3</sup>

The phyllode theory seems completely to explain the anatomy of the isobilateral equitant leaf; in this respect a comparison with the bundle system of various *Acacia* phyllodes proves illuminating. The comparison of an *Iris* leaf, with its two opposed series of bundles, to an *Acacia* phyllode has, indeed, been frequently made,<sup>4</sup> but no one seems to have hitherto taken the view that the similarity which they show yields the clue to the interpretation of the isobilateral equitant leaf. The flattening of a petiole in the vertical plane can scarcely take place without the production of two opposed series of bundles; Fig. 2 B-D, p. 474, shows the actual anatomical effect of flattening and expansion in successive petioles of the seedling of *Acacia nerifolia*. The phyllode of this *Acacia* is closely comparable in venation and anatomy with the leaf of *Tritonia*<sup>5</sup> (Iridaceae, Fig. 15 A-D, p. 483). In both *Acacia nerifolia* and *Tritonia* there is a specialized region, which the writer proposes to call a 'pseudo-midrib' (*p.m.r.*), in which the two main laterals (*m.l.*) are concerned. Such a pseudo-midrib is a common character of the Tribe Gladioleae,<sup>6</sup> to which *Tritonia* and *Gladiolus* belong, while it occurs also in a slightly modified form in some species of *Iris* of the Section *Tetragonae*.<sup>7</sup> Outside the Araceae it is found in *Acorus* (Araceae, Fig. 18 A and B, p. 483). In many other isobilateral equitant leaves no such distinct pseudo-midrib is evident, e. g. *Tofieldia* (Liliaceae, Fig. 20 A-C, p. 483), *Anigozanthos* (Amaryllidaceae, Fig. 19 A and B, p. 483), and species of *Iris* not included in the Section *Tetragonae*, such as that

<sup>1</sup> Chodat, R., and Balicka-Iwanowska, G. (1892).

<sup>2</sup> Balicka-Iwanowska, G. (1892-3).

<sup>3</sup> The present writer hopes to deal more fully with the morphology and anatomy of the leaves of the Iridaceae in a later paper, so all discussion of such forms as *Crocus*, *Romulea*, *Cipura*, &c., is omitted here.

<sup>4</sup> e. g. Bower, F. O. (1888).

<sup>5</sup> The species examined and figured here was a garden hybrid, probably between *Tritonia crocata*, Ker-Gawl., and *Montbretia polystii*, Baker.

<sup>6</sup> Chodat, R., and Balicka-Iwanowska, G. (1892).

<sup>7</sup> Balicka-Iwanowska, G. (1892-3).

represented in Fig. 17 A and B, p. 483; these cases are best compared with such Acacias as *A. cyclops* (Fig. 21, p. 483).

Figs. 15–20, p. 483, illustrate the essential similarity between isobilateral, equitant leaves belonging to different Families. The main anatomical difference between them depends on whether the bundles in the flattened region are opposite one another or alternating. In the former case they sometimes fuse to double bundles with a single xylem and two phloem groups (*Tritonia*, Fig. 15 C, and *Tofieldia*, Fig. 20 C). If they alternate, the thinner part of the flattened leaf may contain a single series of bundles, some with their xylem directed towards one face and some towards the other (*Anigozanthos*, Fig. 19 B, and *Iris* sp., Fig. 17 B). In the case of *Tritonia*, a slight complication is introduced by the production of a double bundle through the branching of the median strand<sup>1</sup> (Fig. 15 A–C), and the development of a wing to the midrib. But these variations are unimportant from our standpoint, and do not detract from the essential uniformity of the anatomical type.

A problem which seems to demand a solution is why, in Monocotyledons, we only meet with leaves flattened in the vertical plane in cases where the phyllotaxis is distichous. The answer probably is that, in the case of a leaf with the broad sheathing base characteristic of a Monocotyledon, vertical flattening is almost a physical impossibility if the leaves are arranged in more than two ranks. For if the main part of the leaf is flattened vertically, the sheath, at least in its upper region, must necessarily be correspondingly flattened, and hence the base of the next leaf can only be fitted in, if it be placed exactly opposite to the first. In other words, we may say that, amongst Monocotyledons, the development of a vertically flattened phyllode is conditioned by a distichous leaf arrangement, while in *Acacia* the narrow attachment to the axis renders the leaf independent of any special type of phyllotaxis. The opportunity for the development of a vertical phyllode must have frequently occurred among Monocotyledons, for distichous phyllotaxy is noticeably widespread in this Class. In addition to those Families already enumerated, in which—if we may so express it—advantage has been taken of the distichous habit to develop a vertical phyllode, there are numerous other cases of two-ranked leaves. Distichy is common amongst the Amaryllidaceae, Scitamineae, Gramineae, Potamogetonaceae, Typhaceae, and Sparganiaceae, while cases occur in the Liliaceae, Juncaceae, Hydrocharitaceae, Pontederiaceae, and Centrolepidaceae. We may conclude that the association of the vertical leaf with distichy does not in any way invalidate the phyllode interpretation; it may, however, seem to the supporters of the ‘concrecence theory’ in some respects favourable to their view.

A case which might perhaps serve to support the ‘concrecence

<sup>1</sup> Chodat, R., and Balicka-Iwanowska, G. (1892).

theory' (though the present writer is not aware that it has ever been so used) is that of *Phormium tenax*, Forst. The leaf of this plant is vertically flattened for a short distance, but opens out above into an expanded portion. It is possible, however, that we have here an instance of an isobilateral leaf which has developed a 'pseudo-lamina' at its apex. It is perhaps significant that, though most of the other *Hemerocallineae* have no lamina, one genus, *Hosta* (*Funkia*), is characterized by possessing a blade whose venation distinctly suggests that it may have been developed by an expansion of the upper part of the petiole (Fig. 22).

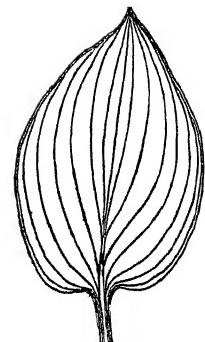


FIG. 22. *Funkia grandiflora*, Sieb. and Zucc. Upper part of petiole and 'lamina' to show venation (reduced).

### 5. Cases of 'phyllodic' anatomy which occur among Dicotyledons and the question of adaptive interpretations.

The anatomical argument in the present paper is based upon the assumption that the occurrence of inverted bundles, which characterizes the leaves of a large number of Monocotyledons, is a feature which is normally absent from the laminae of Dicotyledons (excluding the principal ribs). If it were a common characteristic of both Dicotyledons and Monocotyledons, the contentions here advanced would fall to the ground. It is, of course, well known that the vast majority of Dicotyledonous laminæ exhibit, in transverse section, only a single series of bundles. But it might possibly be argued that extra, inverted bundles are not an ancestral feature, as here maintained, but represent a structural adaptation, and might thus be expected to occur in Dicotyledons as well as Monocotyledons if the thick or succulent nature of the leaf rendered such a development advantageous. It is not possible here to treat this question at length, but it may be pointed out that many succulent leaves among the Dicotyledons, and some among Monocotyledons, are furnished with normally orientated bundles only, so that there is obviously no inevitable connexion between succulence and inverted bundles. Certain Dicotyledonous leaves, on the other hand, in which the anatomy is more or less radial and a peripheral series of bundles occurs, may possibly be interpreted either as laminae reduced to the midrib alone, or as true petiolar phyllodes.<sup>1</sup> There is no difficulty in supposing that phyllodic leaf-structure may have arisen more than once in the phyletic history of the Angiosperms—though never with such far-reaching consequences as when it appeared in the ancestral Monocotyledon.

<sup>1</sup> The writer hopes to consider certain of these cases in a later paper, as well as the various types of reduced leaf found in the perianth of the Angiosperms.

Instead of regarding a peripheral series of bundles as an adaptation developed in response to the succulent and xerophilous habit, the present writer looks upon the existence of such bundles as an indication that the leaf in question is morphologically equivalent to a petiole, or to a petiole and midrib; but, since peripheral bundles happen to be adapted to the requirements of a succulent leaf, they may well have been one of the factors concerned in rendering a xerophilous habit possible. On this view, the 'radial' leaf, whether it belongs to a Dicotyledon or Monocotyledon, is regarded as owing its form and structure *primarily* to its morphological nature, the adaptational use of its structural peculiarities being entirely secondary.

It may be added that it seems scarcely possible to suppose that the inverted series of bundles in the relatively thin leaf of the Daffodil (Fig. 14, p. 479), for instance, can be of 'survival value' or can represent a special adaptation to the mode of life, while such bundles are absent in the leaf of the Bluebell. The general structure and mode of life of the two plants is closely similar, and the Daffodil does not show any obvious superiority in the struggle for existence. But this point must not be laboured, since it involves the whole vexed question of the meaning of adaptation.

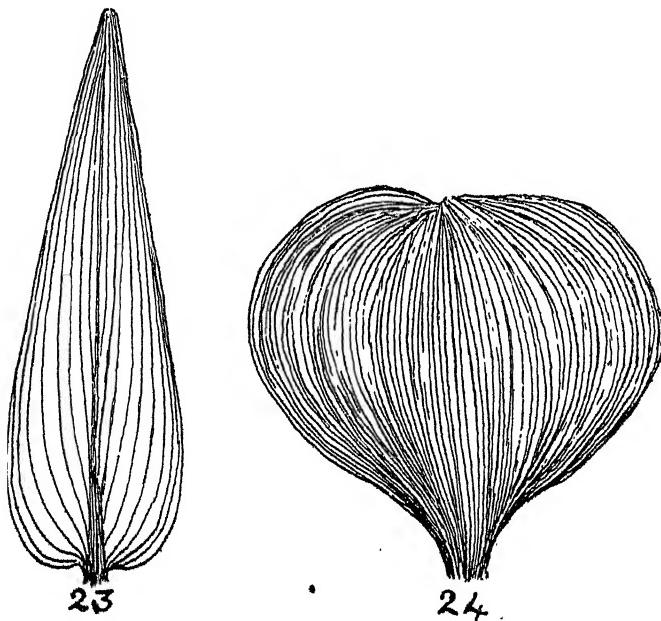
#### 6. The anatomical evidence for Henslow's corollary to the phyllode theory, with special reference to the Pontederiaceae.

Professor Henslow's corollary to de Candolle's phyllode theory has already been outlined (p. 470). He bases his view entirely on external morphology and the macroscopic characters of the venation, but the present writer wishes to draw attention to some cases in which his theory appears to receive definite support from the anatomical structure of the leaves in question.

The Pontederiaceae will be considered in some detail in this connexion, since the peculiarities of their leaf anatomy seem hitherto to have escaped the attention of botanists. The leaves of this Family generally have a sheathing leaf-base, a petiole which is sometimes much swollen, and a 'lamina'. In external appearance and venation the leaves of *Pontederia* (Fig. 23, p. 489) and *Eichhornia* (Fig. 24, p. 489) distinctly suggest that the 'laminæ' are produced by expansion of the apical regions of the petioles, and that they are thus 'pseudo-laminæ' and not equivalent to the blades of Dicotyledonous leaves. The anatomy confirms this idea in a striking fashion. Fig. 28, p. 490, shows the transverse section of a petiole of *Pontederia cordata*, L., with inverted bundles towards the upper side. When the 'lamina' is cut transversely, its structure is found to be exactly such as might have been anticipated on the theory that it is produced by extreme flattening and expansion of the petiole in the horizontal plane (Fig. 26,

p. 490). For, instead of the bundles all being orientated with the xylem upwards, as is usual in laminæ, the vascular strands, though in a single series, are orientated, some normally (*n.b.*) (including the median bundle *m.b.*), the majority inversely (*i.b.*), and a few obliquely (*o.b.*). A small part of the transverse section is shown in greater detail in Fig. 27, p. 490. In this drawing the central and largest bundle is seen to be normally orientated, but the bundles on either side of it have the xylem below and phloem above.

In the heart-shaped 'lamina' of *Heteranthera reniformis*, Ruiz and

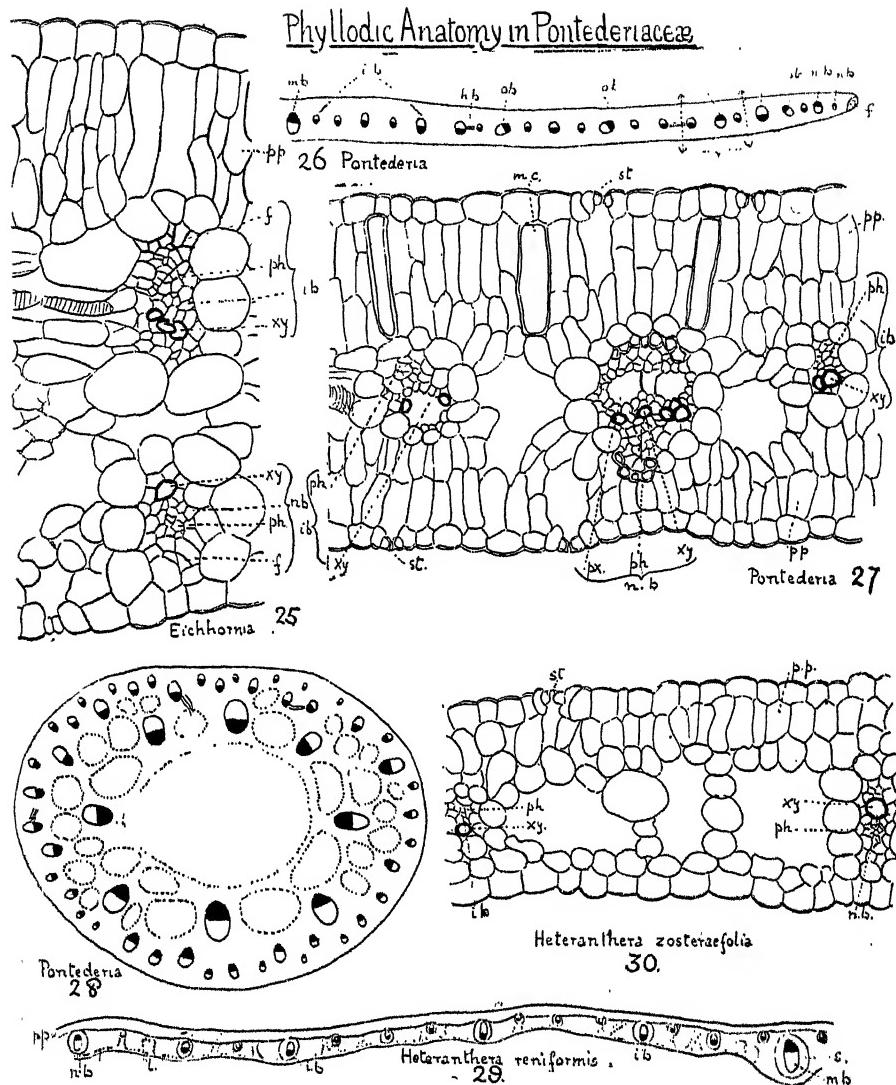


FIGS. 23 and 24. Fig. 23, 'Lamina' of *Pontederia cordata*, L. (natural size), to show venation.  
Fig. 24. A small 'lamina' of *Eichhornia speciosa*, Kunth (natural size), to show venation.

Pav., a very similar bundle arrangement is found (Fig. 29, p. 490). Here only the midrib and main laterals are normally placed, the remaining bundles being inverted.

The orientation of the bundles in these and other members of the Pontederiaceæ closely recalls the arrangement in the leaf of *Iris* shown in Fig. 17 B, p. 483, and also that in the thinner marginal regions of the isobilateral leaf of *Anigosanthos* (Fig. 19 B, p. 483), but the flattening in these cases takes place in the vertical and not in the horizontal plane.

The 'lamina' of *Eichhornia speciosa*, Kunth (Fig. 24), differs from that of those other members of the Family here considered, in its much greater thickness. Inverted bundles occur, not only in the thick basal region—in



FIGS. 25-30. Pontederiaceae. Fig. 25. *Eichhornia speciosa*, Kunth. Transverse section of lateral vein of 'lamina' ( $\times 200$  circa). One small normal bundle (n.b.). One larger inverted bundle (i.b.), higher in leaf, is giving off a branch, also inverted. f. = fibres; ph. = phloem; xy. = xylem. Fig. 26. *Pontederia cordata*, L. Half transverse section of lamina near apex ( $\times 23$ ). All bundles inverted (i.b.) or oblique (o.b.) except the median bundle (m.b.) and the three bundles n.b., n.b.', and n.b''. Fibres (f.) at margin; h.b. = horizontal branch. Fig. 27. *Pontederia cordata*, L. The part of the transverse section shown in Fig. 26 which is included between the dotted arrows ( $\times 200$  circa). One normal bundle (n.b.) and two inverted bundles (i.b.), one with an inverted branch. m.c. = cells containing a secretion, probably myriophyllin. Fig. 28. *Pontederia cordata*, L. Transverse section of petiole near its upper end, outlines of lacunae dotted ( $\times 23$ ). Fig. 29. *Heteranthera reniformis*, Ruiz and Pav. Part of transverse section of lamina, including midrib (m.b.) ( $\times 23$ ). All the bundles shown are inverted, except the midrib and main lateral. s. = bundle sheath. Fig. 30. *Heteranthera zosterifolia*, Mart. Transverse section of part of ribbon leaf ( $\times 200$  circa), to show one normal and one inverted bundle.

which the transition from petiole to 'lamina' takes place quite gradually—but also near the margin. Here there is only a single series of vascular strands, among which inversely orientated bundles are very numerous. Some of the lateral veins in the 'lamina' consist of a single, normally orientated bundle, while others consist of a pair of bundles, one normal and one inverted (Fig. 25, p. 490).

Among the Pontederiaceae, we not only find leaves, such as those just described, in which there is a differentiation between petiole and 'lamina', but others, which are ribbon-like, with no distinction of blade and stalk. For comparison with the more highly differentiated types, sections were cut of the ribbon-leaf of *Heteranthera zosterifolia*, Mart. Here the midrib and main laterals proved to be normal, but the others—i. e. the majority of the laterals—were inverted. Fig. 30, p. 490, shows two adjacent bundles orientated in opposite ways. The structure of this ribbon-leaf is closely similar to that of the 'lamina' in *H. reniformis*.

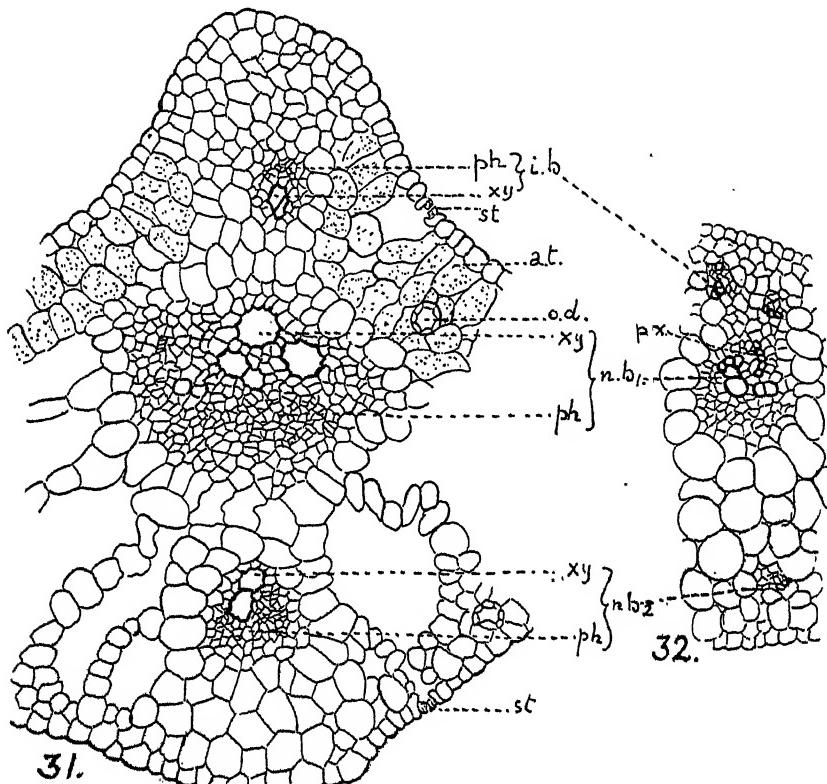
It may be worth noting that a peculiar submerged member of this Family, *Hydrothrix Gardneri*, Hook. f., described by Goebel,<sup>1</sup> has, on its long shoots, leaves with a sheathing base and hair-like upper region, whose external morphology distinctly suggests a phyllodic origin. Anatomical evidence cannot be looked for here, since the extremely slender leaves are said to be traversed by a single bundle only.

The presence of inverted bundles in four species of Pontederiaceae representing three genera—in fact, in all the species of which material has been available to the present writer—is a remarkable anomaly which calls for some explanation. It is difficult to see how such a structural peculiarity can be explained as an adaptation, since it is common to leaves otherwise differing notably in type and mode of life. It is equally conspicuous in the very delicate ribbon-leaf of *Heteranthera zosterifolia* and in the well-defined, thick 'lamina' of *Eichhornia speciosa*; it occurs both in *Heteranthera reniformis*, in which palisade parenchyma is confined to the upper side, and in *Pontederia cordata*, in which this tissue is developed towards both surfaces. In the present writer's opinion, this anatomical anomaly is best interpreted on the view that the 'laminæ' of the Pontederiaceae, instead of being homologous with the laminæ of Dicotyledons, are merely the expanded apices of pre-existing phyllodes: the inverted bundles are thus an indication of the petiolar nature of the organ, and are regarded as an ancestral feature rather than as an adaptation.

But even if the probability of the truth of the phylloide interpretation be admitted for the 'lamina' of the Pontederiaceae, botanists may prefer to regard this group as possessing a unique leaf structure from which no conclusions can be drawn regarding other Monocotyledons. The Pontederiaceae are not, however, the only Family in which we meet with

<sup>1</sup> Goebel, K. (1913).

phyllodic anatomy of the 'lamina'. The present writer has found in the arrow-head blade of *Sagittaria montevidensis*, Cham. and Schlecht. (Fig. 11, p. 479, and Fig. 32), that, besides the normal main bundles (*n.b.* 1) and a series of smaller bundles running near the lower surface (*n.b.* 2), there is a third series of small *inverted* bundles near the upper surface (*i.b.*).



Figs. 31 and 32. *Sagittaria*. (*n.b.* 1 = bundle of main normal series; *n.b.* 2 = bundle belonging to second normal series; *i.b.* = inverted bundle; *xy* = xylem; *ph* = phloem; *a.t.* = assimilating tissue; *st* = stoma; *o.d.* = oil duct). Fig. 31. *Sagittaria sagittifolia*, L. Transverse section of lateral vein of lamina, next but one to midrib ( $\times 400$  circa). Fig. 32. *Sagittaria montevidensis*, Cham. and Schlecht. Small part of transverse section of leaf near margin in region between arrows in Fig. 11, p. 479 ( $\times 100$  circa). The lower of the two bundles belonging to the normal series (*n.b.* 2) is irregularly placed. Fig. 32 shows that in this genus the inverted bundles are not confined to the ribs.

In *Sagittaria sagittifolia*, L., inverted strands are a less striking feature, but the lateral ribs, one of which is represented in Fig. 31, show both normal and inverted bundles.

The present writer has not yet succeeded in finding inverted bundles in the blades of any other Family, except the Hydrocharitaceae, in which their existence was already known. In this Family such bundles occur not only in the undifferentiated leaves of *Enhalus*<sup>1</sup> and *Stratiotes*,<sup>2</sup> but also in

<sup>1</sup> Magnus, P. (1870).

<sup>2</sup> Solereder, H. (1913).

the aerial and floating 'laminae' of *Hydrocharis*, *Limnobium*, and *Hydro-mystria*.<sup>1</sup> Solereder, to whose work on this Family we have already referred, regards the Hydrocharitaceae as unique in this respect, but the present writer has, as has just been shown, found comparable cases in the Pontederiaceae and Alismataceae. As we have already pointed out, Solereder recognizes that the anatomy of the lamina in the Hydrocharitaceae is petiole-like—he says: 'Es ist eben einfach Blattstiel- oder Blattscheidenstruktur'—but he leaves this peculiarity as an unexplained parallelism and draws no conclusion therefrom.

The present writer recognizes that, in spite of the anatomical evidence here adduced, Henslow's corollary to de Candolle's theory rests on a slenderer basis of evidence than the main theory itself. It is apparently open to us at present to hold one of several alternative views. De Candolle's theory may be accepted, while Henslow's corollary is entirely rejected, or Henslow's view may be accepted as far as the Pontederiaceae are concerned, with the reservation that the 'laminae', occurring elsewhere among the Monocotyledons, may, in some or all cases, have had a different origin. Or, finally, the view may be taken that the Monocotyledons are all descended from a stock in which the lamina had been entirely lost and that the existing Monocotyledonous blades, whether showing inverted bundles or not, are all 'pseudo-laminae' of later development. This is the view held by the present writer, but she realizes that a complete proof is still lacking and that the theory must at present be treated as provisional only.

#### 7. Certain obscure anatomical features of Monocotyledonous leaves and their interpretation on the phyllode theory.

##### (i) Strands of fibres.

In some Monocotyledonous leaves, besides the vascular bundles, longitudinal strands occur consisting exclusively of fibres. *Potamogeton zosterifolius*, Schum., furnishes a striking instance of this. One of the figures given by Raunkiær<sup>2</sup> of the leaf of the winter bud of this species shows, in transverse section, a midrib and four lateral vascular bundles situated about half-way between the upper and lower surfaces of the leaf, while, in addition to a thick strand of fibres close to each margin, there is a series of twenty-five fibrous strands near the upper surface and a second series of sixteen fibrous strands near the lower surface. It seems conceivable that these fibrous strands are derived from ancestral vascular bundles, which have lost their conducting tissue and become reduced to fibres alone. The development of fibres in association with the vascular bundles of the leaves of Monocotyledons often occurs on a most remarkable scale; the xylem and phloem in the leaf-bundles of *Agave densiflora* (Fig. 13, p. 479), for instance,

<sup>1</sup> Solereder, H. (1913).   <sup>2</sup> Raunkiær, C. (1896), p. 88, Fig. 49. See also p. 62, Fig. 27 B.

are quite inconspicuous in comparison with the associated fibres. In the cortical bundles of the axis in the Potamogetons, transitions can be traced between the rudimentary vascular bundles enclosed in fibrous sheaths and mere bast bundles.<sup>1</sup> If the fibrous strands of the leaf of *Potamogeton sosterifolius* are really reduced bundles, it is possible that the leaf originally corresponded in anatomy to such a leaf as that of *Narcissus pseudo-narcissus* (Fig. 14, p. 479). If that be the case, the fibrous strands may be regarded as a masked and vestigial indication of phyllodic structure. This suggestion is, however, highly tentative, especially as there appears to be no means, in the case of strands of fibres, for determining the orientation of the pre-existing vascular bundle. Whether this suggestion can be extended to other plants must, at present, remain doubtful; it is probable that fibrous strands may have different origins in different cases.

#### (ii) Apical openings.<sup>2</sup>

Openings in the epidermis and subjacent tissues at the apex of the leaf, by means of which the tracheides, in some cases, come to be actually exposed, occur in many submerged plants. In the case of Dicotyledons, these apertures generally arise through the decay and disintegration of water stomates or the disarticulation of an apical trichome. But in the case of a number of Monocotyledons, the pores arise merely by the general destruction of the apical tissues involved, without the loss of any definite organ. The present writer wishes to suggest that the tendency to the formation of these openings may, in the case of Monocotyledons, be associated with the ancestral loss of the lamina and the consequent 'unfinished' condition—if we may so express it—of the apical region of the leaf. The tendency of the veins in phyllodes to converge towards the tip might, not improbably, lead automatically to the formation of an apical complex of tracheides, such as we often find associated with a terminal opening. These apical pores are generally regarded as adaptations for maintaining the current through the plant by forming a passage for the elimination of water. No doubt, in some instances they perform this function, but there are other cases, e. g.: *Hydrocleis nymphoides*, Buchen., in which, though the apical cavity exists, it remains permanently roofed in with cuticle.<sup>3</sup> It is obvious that this arrangement cannot be explained as an adaptation for the emission of water, but it is possible that it indicates an inherent state of the leaf, which, in other plants, has come to subserve a physiological function.

<sup>1</sup> Raunkiær, C. (1903).

<sup>2</sup> See Sauvageau, C. (1891), Weinrowsky, P. (1899), Minden, M. von (1899), &c.

<sup>3</sup> Sauvageau, C. (1893).

8. The significance of the systematic distribution of phyllodic leaf anatomy among the Monocotyledons.<sup>1</sup>

The two Monocotyledonous Cohorts, in which phyllodic leaf anatomy is at present most widely known, are the Helobiae and the Liliiflorae. In the Helobiae phyllode structure has been found in five Families out of seven, while in the Liliiflorae it has been recorded in six Families out of eight. It is a striking fact that—if the theory be accepted that the Monocotyledons are descended from the Ranalean plexus<sup>2</sup>—it is these two Cohorts which will probably be regarded as including the members of the Class which have retained the greatest number of primitive features. Within the Liliiflorae we find phyllodic characters conspicuously developed in the central group—the Liliaceae. Within the Liliaceae the most striking feature in the distribution of phyllodic structure is that examples are known from no less than six tribes of the Asphodeloideae. This gains significance from the fact that the type of seedling structure occurring in *Anemarrhena*, a member of the Asphodelaceae, has been regarded by Miss Sargent<sup>3</sup> as probably ‘primitive among Monocotyledons in general as well as among the Liliaceae proper’.

The Juncaceac, Amaryllidaceae, Haemodoraceae, and Iridaceae show every indication of being Families derived from the Liliaceous stock. They have, in many cases, retained the phyllodic anatomy which the present writer regards as a primitive character among the Liliaceae. In the Iridaceae it is perhaps more conspicuously developed than in any other Family, and this fact may possibly be taken to indicate that the Iridaceae arose from the Liliaceous stock at a period when that stock exhibited the phyllodic character even more markedly than it does at the present day. At the risk of seeming far fetched, it may be suggested that the behaviour of the Iridaceae in this respect is comparable with the fact that the American descendants of the Pilgrim Fathers, who left this country in the seventeenth century, have retained a few archaic forms of speech characteristic of the England of that period.

Among the Spathiflorae, phyllodic anatomy is, at present, known only in the genus *Acorus* of the Araceae (and probably in the related genus *Gymnostachys*). It would be unsafe to lay much stress upon this individual case, but it is interesting to note that in *Acorus* the flower is hermaphrodite with free perianth members and with the five trimerous whorls characteristic of typical Monocotyledons. The Pothoideae, to which it belongs, are regarded by Engler<sup>4</sup> as representing the oldest Family of the Cohort. It may also be recalled that Miss Sargent<sup>3</sup> considered that the Araceae could be related by seedling characters to the Liliaceae.

<sup>1</sup> In connexion with this section see the list of cases, pp. 478–81.

<sup>2</sup> See Sargent, E. (1903), &c., and Arber, E. A. N., and Parkin (1907).

<sup>3</sup> Sargent, E. (1908).

<sup>4</sup> Engler, A. (1889).

The position of the Farinosae is somewhat doubtful, but the Family Pontederiaceae—in which, as we have shown (pp. 488–91), phyllodic characters are conspicuous in the leaf anatomy—exhibits considerable resemblance to the Liliiflorae. At present no other instances of phyllodic anatomy are known with certainty from the Farinosae. It is, however, not unlikely that the awl-like leaves of certain of the Restiaceae—unless their anatomy is too reduced—might reveal this type of structure on examination, and it is also probable that the vertical leaves of *Anarthria*<sup>1</sup> belonging to the same Family, and those of the Xyridaceae and Philydraceae, would prove to conform to the *Iris* type. The writer hopes in the future to test these suppositions.

Phyllodic leaves of the isobilateral equitant type occur, though rarely, in the Orchidaceae (*Microspermae*).

At present there appears to be no record of the type of structure here called phyllodic, from any other Cohort of Monocotyledons, but, until the Class has been exhaustively examined from this point of view, it is obvious that this result cannot be accepted as final. It may be useful, however, to take stock of the present position, while recognizing the inevitable incompleteness of the data.

The Cohorts in which phyllodic anatomy is at present unknown are the Pandanales, Glumiflorae, Principes, Synanthes, Scitamineae, and Triuridales. On the theory that the Monocotyledons are descended from Ranalean ancestors, these Cohorts all seem to represent groups which have, on the whole, departed widely in vegetative characters from the original Monocotyledonous stock.

We are thus led to the general conclusion that, so far as our present knowledge goes, phyllodic leaf anatomy is most common in those Cohorts of the Monocotyledons which, in other respects, seem to retain primitive characters. This conclusion appears to the present writer to afford some slight indirect support to the phyllode theory. If the ancestral Monocotyledon possessed a phyllode which performed the same functions as those of a Dicotyledonous blade, it is conceivable that the leaves of its more advanced and modified descendants might eventually lose those vestigial, anatomical characters which originally branded them as petiolar, and that they might ultimately approximate by homoplastic convergence to the structure of a Dicotyledonous leaf. There is also a second phyletic course which may in some cases have led to the loss of phyllodic characters. This is the occurrence of a further degree of reduction in the leaf, involving the disappearance of the petiolar portion and the retention of the leaf-base alone; in the latter region inverted bundles are characteristically absent.

<sup>1</sup> Brown, R. (1810).

## III. SUMMARY.

## I. External Morphology.

The first part of the present paper opens with a discussion and amplification of de Candolle's theory of the Monocotyledonous leaf and of Henslow's corollary to that theory. According to de Candolle's theory, the typical Monocotyledonous leaf is interpreted as equivalent to the leaf-base and petiole alone of a Dicotyledon, but the present writer regards certain Monocotyledonous leaves as having been still further reduced until they are equivalent to *leaf-bases* only. Henslow's corollary explains the 'lamina' of those Monocotyledonous leaves, which show a distinction between petiole and blade, as being merely an expansion of the apical region of the original phyllode and thus not homologous with the lamina of Dicotyledon; the present writer proposes to call such a blade a 'pseudo-lamina' (p. 470). It is pointed out that these theories explain the venation of Monocotyledonous leaves (p. 467).

It is shown that the phyllode theory is in no way inconsistent with Miss Sargent's hypothesis of the geophytic nature of the original Monocotyledonous stock. As regards the embryo, the present writer proposes the further corollary that the single cotyledon of the Monocotyledon is equivalent only to the fused bases and petioles of the Dicotyledonous seed-leaves, the cotyledonary laminae being unrepresented (p. 468).

Asa Gray's tentative suggestion that some Gymnosperm leaves might be equivalent to petioles is recalled and expanded, and the writer suggests its special application to the case of the Gnetales. It is pointed out that on the phyllode theory the Coniferae would be regarded as microphyllous by reduction (p. 472).

## 2. Anatomy.

The phyllode theory has hitherto been based entirely on external morphology, but, in the second part of this paper (p. 473), reason is given—on the ground of a comparison of Dicotyledonous scale-leaves, petioles, and phyllodes with the leaves of Monocotyledons—for the view that the occurrence of inverted vascular bundles, towards the adaxial face of a leaf, may be an indication of phyllodic morphology. A list is added of the cases of such structure in Monocotyledons (pp. 478–81). In most of the cases to which this list relates, the facts of the anatomy were already known, but no one hitherto appears to have regarded the occurrence of these inverted bundles as furnishing—in correlation with the external form—the key to the morphological interpretation of the Monocotyledonous leaf.

The isobilateral equitant leaf of *Iris*, &c., is regarded by the present writer, not as exhibiting congenital concrescence of the two halves of the organ, but

as a special case of phyllodic structure (p. 482). It is shown that this type of leaf is widely distributed among the Monocotyledons, that it can be closely compared with certain *Acacia* phyllodes, and that transitions can be traced between it and other types of Monocotyledonous phyllode.

It is suggested that certain examples of 'radial' leaf anatomy among Dicotyledons may possibly be explained on lines similar to those indicated for the case of 'phyllodic' Monocotyledons. It is proposed that, in lieu of regarding a centric leaf with peripheral bundles as an adaptation to xerophilous life, it may be more logical to interpret this type of anatomy as an hereditary indication of the phyllodic (or midrib) nature of the leaf; that it happens to be one of the features which may enable the plant possessing it to become a xerophyte, is considered to be merely a secondary result.

Certain tentative and provisional suggestions are made (p. 493) regarding the interpretation, upon the phyllode theory, of the significance of strands consisting exclusively of fibres, and also of the origin of the 'apical openings' occurring in the leaves of some submerged Monocotyledons.

Anatomical evidence is then brought forward in favour of Henslow's corollary to de Candolle's theory (p. 488). It is shown, apparently for the first time, that the 'laminae' of certain Pontederiaceae (*Eichhornia*, *Pontederia*, *Heteranthera*) all agree in the presence of inverted as well as normal bundles. Inverted bundles are also recorded in the lamina of *Sagittaria*. Attention is drawn to Solereder's discovery of inverted bundles in the 'laminae' of certain Hydrocharitaceae. It is recognized that Henslow's corollary depends upon a more slender basis of evidence than the main theory, but the present writer is disposed to consider that it is well founded and that the blades of Monocotyledonous leaves are all, in reality, 'pseudo-laminae'.

The systematic distribution of phyllodic anatomy among the Monocotyledons is then dealt with (p. 495), and it is shown that, as far as our present knowledge goes, it does not occur with any frequency outside the Helobiae, Liliiflorae, and Farinosae. On the theory of the origin of the Monocotyledons from the Ranalean plexus—which is accepted by the present writer—the Helobiae and Liliiflorae include those members of the Class which have departed least in character from the ancestral stock; the Farinosae are probably not far distant from the Liliiflorae. In the case of the Spathiflorae, the only record relates to *Acorus*, which is probably the member of the Araceae retaining the most primitive floral characters—but this may be a mere coincidence. The Cohorts which rarely or never display phyllodic anatomy are those which, on other grounds, are regarded as advanced and highly modified. It is concluded that the systematic distribution harmonizes with the view that the type of anatomy here called 'phyllodic' is an ancient character, revealing the petiolar origin of the Monocotyledonous leaf.

## ACKNOWLEDGEMENTS.

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# Additional Notes upon the Angiosperms *Tetracentron*, *Trochodendron*, and *Drimys*, in which Vessels are absent from the Wood.

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With Plate XVI and nine Figures in the Text.

In a paper read at the celebration of the twentieth anniversary of the New York Botanical Garden, September 1915, the writers (11) discussed the significance of certain features in the anatomy of *Tetracentron*, *Trochodendron*, and *Drimys*. It was shown that not only are true vessels entirely absent from the normal wood of the stem of these genera, but also from those organs or regions<sup>1</sup> that are considered by certain morphologists to be retentive of ancestral characters.

The accuracy of the conclusions reached by the writers in regard to the absence of vessels in *Tetracentron*, *Trochodendron*, and *Drimys* has been brought into question by the work of Jeffrey and Cole (6). These investigators claim to have found vessel-like structures in injured roots of *Drimys colorata*, which abnormalities are considered to indicate that the ancestors of *Drimys*, *Trochodendron*, and *Tetracentron* possessed true vessels.

However, it is admitted by Jeffrey and Cole that the elements, described and figured by them, lack the perforations of normal vessels. In view of this fact, it is desirable to determine to what extent these traumatically produced cells are really vessel-like in structure.

The word vessel, which was used in a variety of meanings by the older phytotomists, was first clearly defined by von Mohl (8) in the middle of the last century.

'The primary form of the elementary organs of plants is that of a completely closed, globular or elongated vesicle, composed of a solid membrane

<sup>1</sup> Root, leaf, node, floral axis, seedling, first annual ring, &c.

and containing a fluid. If this remains still closed after its development is completed, it is called a cell, *cellula*, but if a row of utricles arranged in a line become combined, during development, into a tube with an uninterrupted cavity, through the absorption of their cross walls, a compound elementary organ is produced—the vessel (spiroid of Link).<sup>1</sup>

The importance of von Mohl's developmental studies was appreciated by many of his contemporaries, and his distinction between vessels, compound structures, and simple tracheary cells has been accepted, in but slightly modified form, by subsequent writers, including Caspary, Sanio, de Bary, Sachs, Strasburger, Van Tieghem, Scott, and Coulter.

Thus, in its generally accepted modern meaning, a vessel, *Gefäß*, *trachea* (Sanio), is a compound structure that arises from a series of cells by the loss of the pit membranes in the division walls between the members of the series, the latter being termed the members or segments of the vessel.<sup>1</sup>

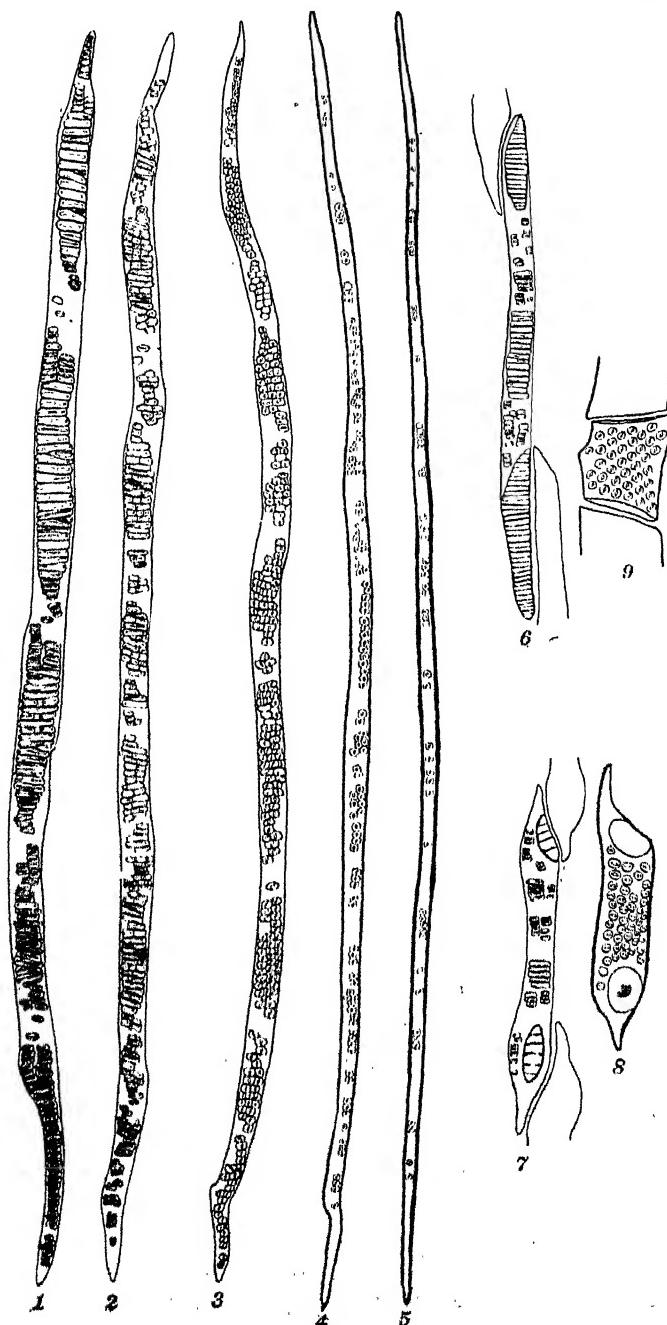
The traumatically produced structures, described and figured by Jeffrey and Cole, are not compound structures, but simple cells, having pits with membranes and well-developed bordering areas (Pl. XVI, Fig. 9). Therefore they are not vessel-like in structure.

The next question to be considered is, are these tracheary cells segments of vestigial vessels? If they are to be considered as such, there must be some criterion for distinguishing them from ordinary tracheary elements. In macerations of the secondary wood of plants which possess vessels, it is possible to separate the segments of the vessels from tracheides, fibre-tracheides, and libriform fibres by their size, form, or the structure of their pitted walls. Thus, in the majority of the Dicotyledons, the vessel-segments are not only noticeably larger in diameter than the neighbouring tracheary elements, but show unmistakable evidences of having been joined together to form a segmented tube or 'duct' (Text-figs. 6-9). Even in those exceptional cases where the segments of the vessels resemble the surrounding tracheary cells in general size and shape, they can readily be distinguished from them by the structural peculiarities of certain of their pits, which are perforated and without well-developed bordering areas.

The cells which occur in the root of *Drimys* resemble the surrounding tracheides in general size and shape, and are quite unlike the typical vessel-members of *Euptelca*, *Illicium*, *Kadsura*, *Schizandra*, *Michelia*, *Magnolia*, *Talauma*, and *Liriodendron* (Text-figs. 1-9). Furthermore, these traumatically produced elements possess throughout pits with well-developed bordering areas and membranes, which serve to separate them quite sharply from the vessel-segments of the genera just enumerated.

Jeffrey and Cole placed much emphasis upon the fact that elongated

<sup>1</sup> To refer to a single cell as a vessel, as has been done by Jeffrey and Cole throughout their paper, appears to be somewhat unfortunate, and likely to lead to unnecessary confusion.



TEXT-FIGS. 1-5. Types of tracheides that occur in the secondary xylem of *Tetracentron*, *Trochodendron*, and *Drimys*. 6. Type of vessel-segment that occurs in the secondary xylem of *Cercidiphyllum*, *Euptelea*, and various Monimiaceae. 7. Type of vessel-segment that occurs in the secondary xylem of the Magnolieae. 8. Type of vessel-segment that occurs in the secondary xylem of the Lauraceae and many other Dicotyledons. 9. Type of vessel-segment that occurs

or scalariform bordered pits occurred in the lateral walls of the tracheary structures in the injured roots of *Drimys*.

'We may ask if the peculiar scalariform elements<sup>1</sup> occurring in the root of *Drimys* after injury are in reality to be interpreted as of the nature of vessels. They are certainly not to be considered as tracheides, since the sculpture of their walls is quite unlike that found in tracheides in general, and entirely resembles that observed as characteristic of vessels in the Magnoliaceae and other families.'

However, this conclusion does not appear to be substantiated by well-known facts in the anatomy of the higher plants, since tracheides with scalariform bordered pits occur in many Angiospermae, Gymnospermae, and Pteridophyta.<sup>2</sup> Even in secondary xylems, such tracheides are abundantly developed in certain representatives of the Calamariales, Sphenophyllales, Cycadofilices, and Bennettitales (Pl. XVI, Fig. 11).

In addition, it is to be emphasized that scalariform bordered pits are no more characteristic of the lateral walls of the vessels in the Ranales and other groups of the Dicotyledons than are 'multiseriate' circular types (Text-figs. 8 and 9). It is evident, accordingly, that the presence of non-perforate, scalariform bordered pits cannot be used as a reliable diagnostic criterion for distinguishing vessel-segments.

If the scalariform elements that occur in the roots of *Drimys* are to be considered (phylogenetically) as members of vestigial vessels, it must be admitted that they have lost all the structural peculiarities of vessel-segments and have reverted to typical tracheides. There is no direct evidence in favour of this hypothesis. Nor does the evidence that may be derived from analogy with other Dicotyledons appear to support this view.

As has been noted by Eichler (1), Groppler (3), Harms (4), Solereder (9), Van Tieghem (12), the writers (11), and others, the normal secondary xylem of *Tetracentron* and *Trochodendron* is characterized by the presence of tracheides with scalariform bordered pits (Text-fig. 1, and Pl. XVI, Fig. 6). Although, upon the basis of Jeffrey and Cole's hypothesis, these forms must be considered to be less 'degenerate' than *Drimys*, their scalariform elements are typical tracheides and no more resemble the segments of true vessels than do those which occur in injured roots of *Drimys* (Text-fig. 2, and Pl. XVI, Fig. 9). Therefore Jeffrey and Cole's argument, that 'the general phenomenon of traumatism lead us to expect, more often than not, the recall of ancestral characters in an abnormal form', loses much of its significance.

Although evidences of the partial or nearly complete suppression of vessels occur in a number of Dicotyledons, they occur usually in unusual growth forms, such as aquatics, much reduced parasites, extreme xero-

<sup>1</sup> Referring to elements of the secondary xylem with closed scalariform bordered pits.

<sup>2</sup> The terminology of Engler and Gilg is used in this paper.

phytes, &c., where there are obvious physiological reasons for the degeneration of tracheary tissue. Furthermore, it is a notable fact that, even among such highly specialized plants as these, the complete suppression of vessels does not occur in plants having well-developed secondary xylem in their roots and stems. Poorly developed or vestigial vessels, easily recognized as such by their structural peculiarities, are present in some part of the plant.

However, there does not appear to be any reliable evidence to indicate that *Tetracentron*, *Trochodendron*, and *Drimys* should be placed in this category of specialized Dicotyledons. According to E. H. Wilson (14), the well-known botanical explorer, *Tetracentron sinense*, Oliv., is the second largest Dicotyledonous tree in the montane forests of central and western China. It attains a height of 16–30 m. and a stem circumference of 4–6 m., and has a large crown covered with leaves of extremely herbaceous texture. It grows on moist slopes and rich bottom lands in the neighbourhood of streams. *Trochodendron aralioides*, Sieb. et Zucc., like *Tetracentron*, is a large arborescent form which attains a height of 25 m. and a stem circumference of 3–8 m. It grows in moist, warm, montane forests of central and southern Japan and of Formosa, and has a large crown with well-developed foliage. Unlike these monotypic genera, *Drimys* comprises a number of species which grow in Central and South America, Borneo, New Guinea, New Caledonia, Australia, New Zealand, and Tasmania. They are small trees or shrubs which usually live in relatively moist environments. Their leaves, which vary considerably in size and texture, are not unlike those of many Dicotyledons of moist tropical or warm temperate forests.

In other words, there are no obvious physiological or ecological reasons for the degeneration of vessels in *Tetracentron*, *Trochodendron*, and *Drimys*. From analogy with the structure of other arborescent and fruticose Dicotyledons, it appears to be highly improbable that such a growth form as *Tetracentron*, with its large crown, thin leaves of herbaceous texture, and relatively high rate of transpiration, should once have possessed vessels and subsequently lost them, as has been considered to be the case by Groom (2) and Jeffrey and Cole.

It has been stated by Jeffrey (5) that the so-called laws of recapitulation, reversion, and retention are either of universal validity or of little scientific value, and that they cannot in certain cases be admitted and in others denied. Assuming, for the sake of argument, that such 'laws' can actually be formulated and universally applied in the study of plant evolution,<sup>1</sup> what significance should be attached to the occurrence of scalariform

<sup>1</sup> It should be noted in this connexion that, even if certain organs of plants are inherently more conservative than others, it must frequently be extremely difficult to determine, in the absence of reliable collateral evidence, whether a given structure in a given region is cenogenetic or truly palingenetic; for even the most ardent advocates of the doctrines of recapitulation, reversion, and retention, who tend to minimize or ignore the effects of physiological and ecological factors, admit that cenogenetic characters do occur in roots, seedlings, traumatic tissue, &c.

tracheides in injured roots and first annual rings of stems of *Drimys*? Obviously, these traumatically produced elements might logically be considered to indicate that the ancestors of the existing species of *Drimys* possessed tracheides with scalariform bordered pits, such as occur in the normal secondary xylem of *Tetracentron* and *Trochodendron* (Pl. XVI, Fig. 6), and were present in that of a number of Mesozoic and Palaeozoic plants (Fig. 11).

However, it was appreciated by Jeffrey and Cole that their interpretation of the phenomena in injured roots of *Drimys*—as traumatic recapitulations of ancestral structures in a very ‘conservative’ organ—would lose much of its significance if scalariform elements occurred in the secondary xylem of stems and in uninjured roots. Therefore, much emphasis was placed upon the fact that scalariform tracheary pitting does not occur in stems and uninjured roots except in the immediate vicinity of the primary xylem.

Recently the writers have examined a considerable amount of material of various species of *Drimys*. This material included stems and roots of plants grown in their native habitats in South America and Australasia, and plants grown in nurseries and greenhouses in England and the United States. As is shown in Plate XVI, the tracheides were found to vary considerably in size and in the thickness of their secondary walls. In certain specimens (Figs. 1 and 5), growth layers or concentric rings were clearly differentiated; whereas in others there were only slight indications of zonation (Figs. 2, 3, and 4). In the stems of *D. Winteri*, Forst., secured from small plants grown in greenhouses, and in stems of *D. colorata*, Raoul, and *D. axillaris*, Forst., from New Zealand, the tracheides were provided with one or two rows of circular bordered pits in their radial facets (Text-fig. 4, and Pl. XVI, Fig. 8). On the other hand, in stems of *D. Winteri* from Chile and in roots of *D. colorata* and *D. axillaris* from New Zealand, the larger tracheides tended to have several rows of bordered pits in their radial walls (Text-fig. 3, and Pl. XVI, Fig. 7). Scalariform pits were of more or less frequent occurrence in many of these tracheides (Pl. XVI, Fig. 12). They occurred in normal uninjured stems and roots, and in the latter as well as the earlier formed portions of the xylem. In young stems —showing 5–15 growth layers—of *D. Winteri* from South America, the secondary xylem not infrequently resembled that which occurs in *Tetracentron*. Growth layers were clearly differentiated (Pl. XVI, Fig. 5). The larger, first-formed tracheides in these concentric rings were typical scalariform, whereas the smaller terminal tracheides possessed circular bordered pits (Pl. XVI, Fig. 10). Transitional types of pitting occurred in intermediate tracheides. In other words, the layers of scalariform tracheides, as in *Tetracentron*, were separated by zones of non-scalariform tracheary elements.

It is evident, accordingly, that scalariform pits may occur in the normal

secondary xylem of uninjured stems and roots of *Drimys*. Furthermore, the occurrence and distribution of these structures appears to be influenced, to some extent at least, by purely physiological and ecological factors. Since the occurrence of tracheides with scalariform bordered pits cannot be explained satisfactorily upon the assumption that *Tetracentron*, *Trochodendron*, and *Drimys* are 'evascularized' Dicotyledons—'degenerate' forms whose ancestors possessed true vessels in their secondary xylem—what is the probable significance of these interesting tracheary structures?

From the morphological and phylogenetic points of view, it may be assumed that (1) *Tetracentron*, *Trochodendron*, and *Drimys* are descended directly from ancestors which possessed scalariform tracheides in their secondary xylem, or (2) they are descended from ancestors having tracheides with circular bordered pits. If the latter hypothesis be accepted, the scalariform tracheides in the secondary xylem of *Tetracentron*, *Trochodendron*, and *Drimys* must have been acquired *de novo*, e.g. by the horizontal elongation of single circular bordered pits, by the fusion of rows of circular pits, or by a transfer of the impulse to form scalariform pits from the primary to the secondary meristem—*homoeosis*, Leavitt (7).

In this connexion it is desirable to discuss in greater detail the types of tracheary pitting that occur in *Tetracentron*, *Trochodendron*, and *Drimys*. The secondary xylem in the first two genera is characterized by possessing clearly differentiated growth layers or annual rings (Pl. XVI, Fig. 1). The tracheides in the first-formed portion of the concentric layers are comparatively large and thin-walled and have numerous scalariform bordered pits in the radial facets (Text-fig. 1, and Pl. XVI, Fig. 6); those in the last-formed portion of the rings are small, thick-walled, and fibre-like, and have scattered circular bordered pits (Text-fig. 5, and Pl. XVI, Fig. 6). Between the typical scalariform and fibre-like elements, intermediate types of tracheides with transitional types of pitting occur. These cells show all gradations between scalariform and circular bordered pits. In certain cases the scalariform bordered pits are replaced by two or more smaller pits (Text-fig. 2); in others, by single oval or circular pits (Pl. XVI, Fig. 6). When the former phenomenon occurs, the circular bordered pits form horizontal and vertical rows, so-called 'opposite' pitting. This type of pitting may grade into the type which occurs in the fibre-like tracheides, by a simple process of reducing the number of vertical rows to one, and by a gradual decrease in the number of pits in the remaining row. Occasionally, the vertical rows become more or less 'staggered', producing the so-called 'alternating' type of pitting. In the genus *Drimys* the secondary xylem may be composed of similar combinations of tracheary elements or of one or more of the intermediate or transitional types (Pl. XVI, Figs. 7, 8, 9, 10, and 12).

In so far as the transitional types of pitting, which occur in the secondary

xylem of *Tetracentron*, *Trochodendron*, *Drimys*, and other genera of the Ranales, are concerned, it is just as reasonable to interpret them as indicating that circular bordered pits are derived from scalariform bordered pits as vice versa. Indeed, if one considers that the laws of recapitulation, reversion, and retention cannot in certain cases be admitted and in others denied, the more abundantly developed scalariform pitting in young stems of *D. Winteri* and injured roots of *D. colorata* should be interpreted as indicating that *Tetracentron*, *Trochodendron*, and the various species of *Drimys* form a graded series, illustrating the replacement of scalariform by circular bordered pits. Therefore, in the absence of reliable evidence which might be considered to prove that the secondary tracheides of the Coniferae or similar forms have been or can be modified to form typical scalariform secondary tracheides, the possibility that the vessel-less *Tetracentron*, *Trochodendron*, and *Drimys* are descended directly from ancestors having tracheides with scalariform bordered pits deserves careful consideration in discussions concerning the origin of the Angiosperms.

It is to be emphasized that, although the secondary xylem of *Tetracentron* and *Trochodendron* is in general unlike that of the Coniferae or higher Gymnosperms, it resembles the wood of certain Palaeozoic and Mesozoic plants, e. g. *Calamites*, *Protopitys*,<sup>1</sup> figured by Solms-Laubach (10), and certain Cycadeoideae described by Wieland (18). In all of these plants, as well as in the primary xylem of many ferns, there are transitions between typical scalariform bordered pitting and 'opposite', 'alternate', or uniseriate circular bordered pitting. Recently, one of the writers enjoyed the privilege of studying and photographing Dr. Wieland's excellent slides of type material of *Cycadeoidea Dartoni* (Coulter and Chamberlain), Wiel. As is shown in Pl. XVI, there is a striking similarity between the tracheary pitting of this fossil and that of *Tetracentron* and *Trochodendron*. The writers emphasize this similarity in tracheary structure, not as indicating necessarily close genetic relationship between the Ranales and Bennettiales, but in order to show that certain of the Pteridophyta and older Gymnospermae possessed a more plastic and generalized type of pitting than that of the relatively stereotyped Coniferae.

#### CONCLUSIONS.

The scalariform tracheary elements that occur in injured roots of *Drimys colorata* are not vessel-like in structure.

They are typical tracheides having transitional types of pitting, such as occur in many Pteridophyta, Gymnospermae, and Angiospermae.

They occur in uninjured stems and roots of *Tetracentron*, *Trochodendron*, and *Drimys*, as well as in traumatic tissue.

True vessels do not occur in the xylem of these genera.

<sup>1</sup> This genus is now placed in the Cycadofilices.

Not only is there no direct structural evidence which might be considered to indicate that these vessel-less Angiosperms are 'evascularized' forms, but there appears to be no physiological or ecological evidence for supposing that they are 'degenerate' Dicotyledons whose ancestors possessed true vessels in their secondary wood.

The secondary xylem of *Tetracentron* and *Trochodendron*, which is entirely unlike the wood of the Coniferae, closely resembles that of certain of the Pteridophyta and older Gymnospermae.

In conclusion, the writers wish to thank their botanical colleagues in Australasia, South America, England, and Japan for their courtesy in sending valuable material of *Drimys*, *Tetracentron*, and *Trochodendron*. To Dr. G. R. Wieland and Dr. E. W. Berry the writers are indebted for the opportunity of studying and photographing sections of Palaeozoic and Mesozoic plants.

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## DESCRIPTION OF PLATE XVI.

Illustrating Messrs. Bailey and Thompson's paper on the Angiosperms *Tetracentron*, *Trochodendron*, and *Drimys*.

Fig. 1. *Trochodendron aralioides*. Transverse section of the secondary xylem, showing growth layers.  $\times 90$ .

Fig. 2. *Drimys colorata*. Transverse section of the secondary xylem of a stem.  $\times 90$ .

Fig. 3. *Drimys colorata*. Transverse section of the secondary xylem of a root, showing poorly differentiated growth layers.  $\times 90$ .

Fig. 4. *Drimys Winteri*. Transverse section of the secondary xylem of a mature stem, showing poorly differentiated growth layers.  $\times 90$ .

Fig. 5. *Drimys Winteri*. Transverse section of the secondary xylem of a young stem, showing second, third, and fourth growth layers.  $\times 90$ .

Fig. 6. *Trochodendron aralioides*. Radial longitudinal section of the secondary xylem.  $\times 90$ .

Fig. 7. *Drimys colorata*. Radial longitudinal section of the secondary xylem, illustrating a type of pitting that occurs in the stems and roots of many specimens of *Drimys*.  $\times 90$ .

Fig. 8. *Drimys colorata*. Radial longitudinal section of the secondary xylem, illustrating a type of pitting that occurs in the stems and roots of certain specimens of *Drimys*.  $\times 90$ .

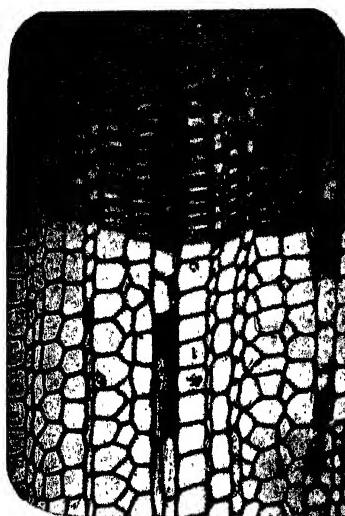
Fig. 9. *Drimys colorata*. Radial longitudinal section of the secondary xylem of an injured root, showing supposed 'vessel-like' structures.  $\times 125$ . (After Jeffrey and Cole.)

Fig. 10. *Drimys Winteri*. Radial longitudinal section of the secondary xylem of a young stem, showing (left) last-formed tracheides of third growth ring, and (right) first-formed scalariform tracheides of fourth growth layer.  $\times 270$ .

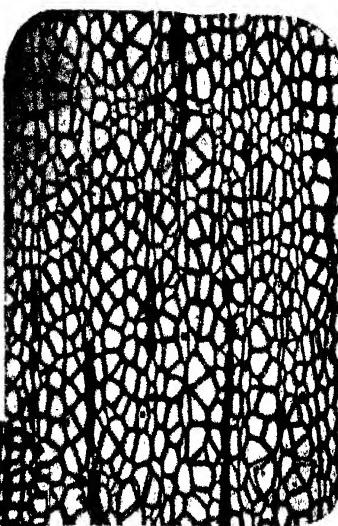
Fig. 11. *Cycadeoidea Dartoni*. Radial longitudinal section of the secondary xylem, showing scalariform and transitional types of bordered pitting.  $\times 100$ .

Fig. 12. *Drimys Winteri*. Radial longitudinal section of the secondary xylem, showing circular and scalariform bordered pits, such as occur in uninjured stems and roots of many specimens of *Drimys*.  $\times 260$ .

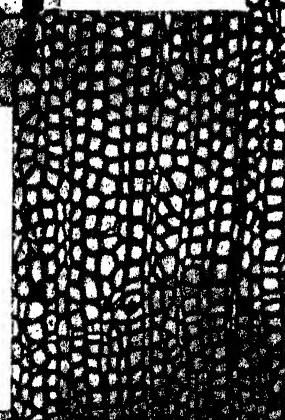




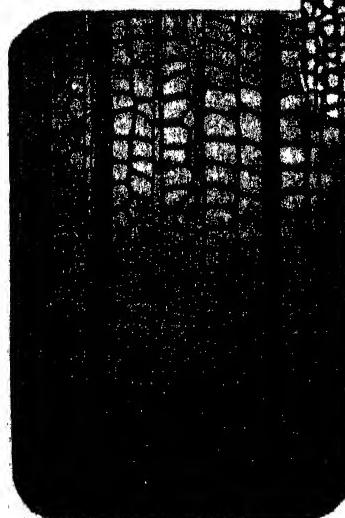
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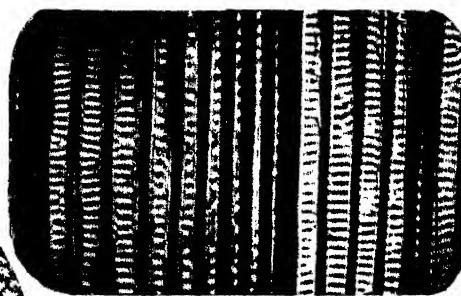
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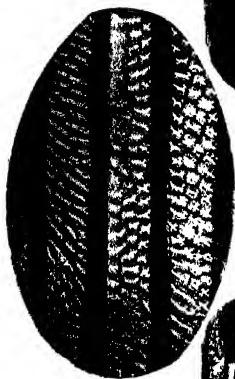
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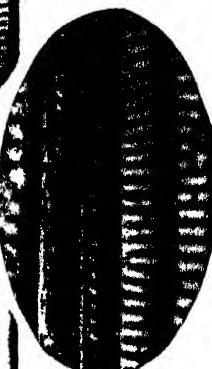
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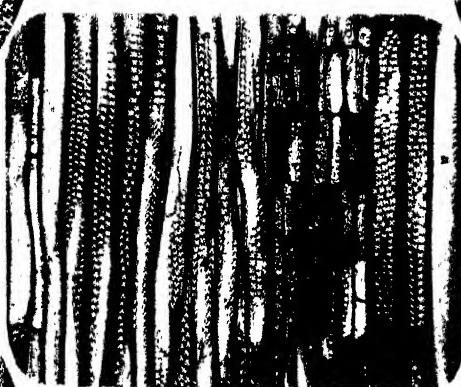
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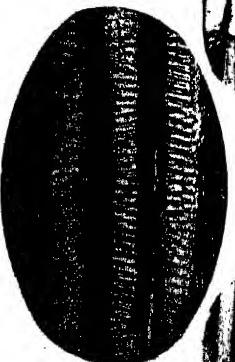
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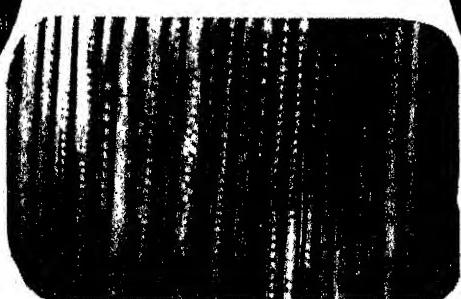
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11.



12.



8.



# Observations on the Anatomy of Teratological Seedlings.

## I. On the Anatomy of some Polycotylous Seedlings of *Cheiranthus Cheiri*.

BY

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With seventeen Figures in the Text.

THE discovery by one of us of a strain of wallflower which threw a considerable percentage of abnormal seedlings seemed to offer a favourable opportunity for an anatomical study of specific teratological polycotylony on a somewhat more extensive scale than had hitherto been possible.

The particular strain of *Cheiranthus*, in addition to producing all types of seedling from the tetracotylous to the normal dicotylous form, exhibited a number of other features of some interest. Among these may be mentioned an alteration in the time of flowering, the production of a rather delicate seedling as compared with more normal strains, and also a relatively high percentage of abortive seeds. Thus in a total sowing of 212 seeds, there were 123 failures, 56 normal seedlings, and 33 abnormal ones.

The number of abnormal seedlings obtained from this source was augmented by the collection of some specimens from a common garden variety, most of these apparently throwing a few polycotyls.

The total number of polycotylous seedlings examined was fifty-four, and these fell into the following groups, as far as one could judge from external characters :

Type of Seedling.	No. of Seedlings.
Hemitricotyls	9
Tricotyls	32
Hemitetracotyls	1
Tetracotyls	12

## METHODS.

The seedlings were uprooted at as early a stage as possible, and were preserved in methylated spirit. Considerable difficulty was experienced in finding a stain suitable for the very delicate seedlings. Although numerous combinations of stains were tried, Delafield's haematoxylin was the only reagent which gave satisfactory results. At a later stage in the investigation, however, when the more robust seedlings of the garden variety were microtomed, good results were obtained with carthamin in 70 per cent. alcohol, followed by Lichtgrün in clove oil.

## DESCRIPTION OF RESULTS.

The structure of the normal seedling of *Cheiranthus Cheiri* has been fully described by Miss Thomas (20), so that no detailed account need be given here.

The slender seedlings show, in every case, a high level of transition.

The base of each cotyledon is occupied by a central 'double' bundle, which in the transition region 'rotates'<sup>1</sup> to form one pole of the diarch root. With this brief summary we may proceed at once to the description of forms showing more or less pronounced polycotyly.

## HEMITRICOTYLY.

The nine seedlings of this type which were examined showed all stages of hemitricotyly, ranging from forms with only a slight notch at the apex of the abnormal cotyledon, to others showing fission right to the base of the lamina (Fig. 1). Usually the two halves of the bifurcated cotyledon were together of approximately the same size as the normal cotyledon (Fig. 1, a), but in one or two instances each half was as large as a whole cotyledon (Fig. 1, b).

One case of considerable interest showed a peculiar asymmetry in the methods of fusion of the two halves of the cotyledon (Fig. 2). One half retained the leaf tissues on both sides of the midrib, whilst the other gradually lost its tissues on the side nearer its fellow, so that at the point of junction of the two halves an irregular Y-shaped structure was formed. This, however, soon became symmetrical by the dying away of the extra flange.

The behaviour of the vascular bundles of the hemitrichotylous seedlings is extremely interesting, and enables one to divide them into two distinct groups.

<sup>1</sup> The terms 'transition' and 'rotation' are used in this paper as convenient conventionalities without implying adherence to the theories of seedling structure in which they originated.

(a) In the seven seedlings comprising the first group the normal cotyledon has a typical double bundle, while the midrib of each half of the bifurcated cotyledon consists of a collateral bundle. Towards the base of the cotyledon, after fusion of the two halves has occurred, the collateral bundles approach one another, while the protoxylems rotate towards the middle line, and fuse so that ultimately the two function as a double bundle,

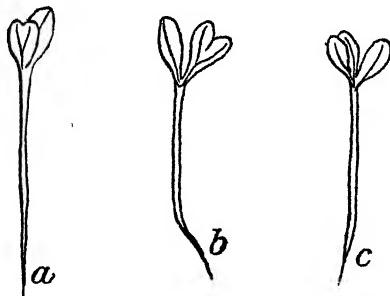


FIG. 1. *a, b, c.* Hemitrichotylous seedlings, showing three stages in the division of the abnormal cotyledon.

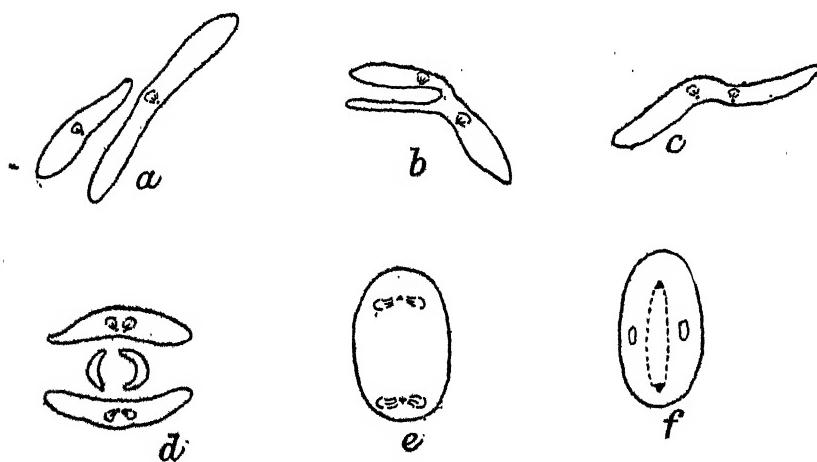


FIG. 2. *a-f.* Hemitrichotyl, Type *a*. Diagrams *a, b, c* show the asymmetrical union of two half cotyledons; the normal cotyledon is not shown. Diagrams *d, e, f* show the structure lower down in this seedling.

producing, together with the double bundle of the normal cotyledon, a typical diarch structure (Fig. 3).

This condition is found in seedlings having the cotyledon split nearly to the base, as well as in those which are merely notched, the only variation being in the level at which the two collateral bundles unite. This union may occur in the lower portion of the cotyledon lamina, in the petiole, or even at the apex of the hypocotyl, but the point of fusion bears no close

relation to the extent of fission, for it may occur at approximately the same level in a cotyledon showing an apical notch, as in one which has a deeply split lamina, the union taking place in both instances in the cotyledon petiole. Even when the two half bundles are a little more widely separated

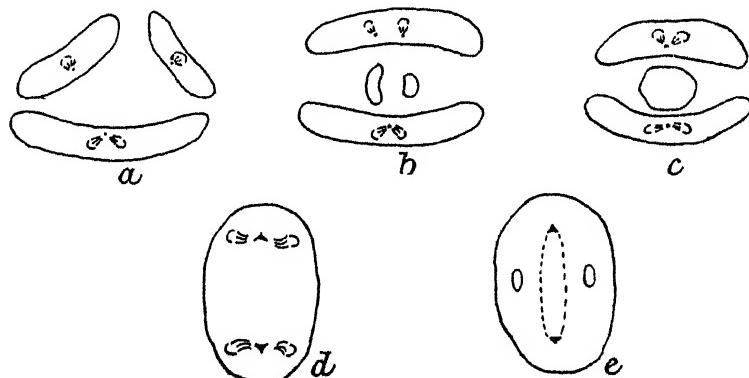


FIG. 3. *a-e.* Hemitricotyl, Type  $\alpha$ . Diagrams showing the characteristic features of this type.

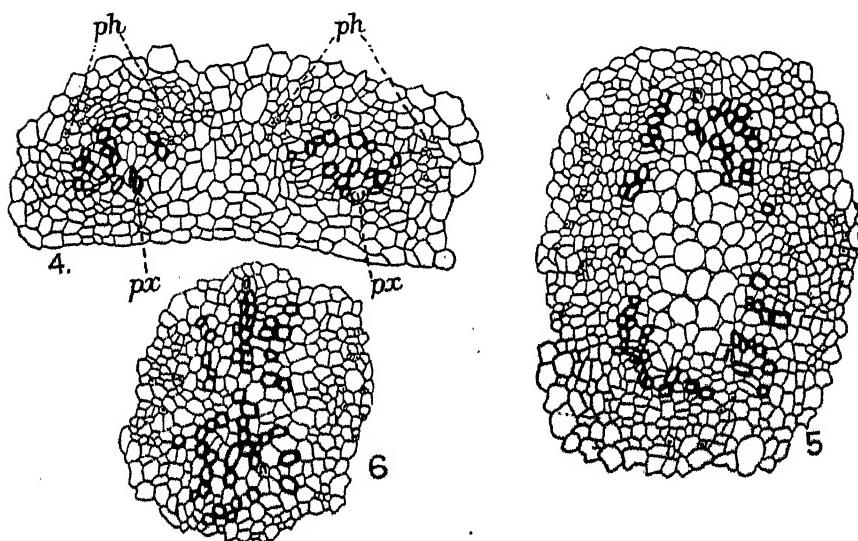


FIG. 4. Hemitricotyl, Type  $\beta$ . Transverse section. Petiole.

FIG. 5. Hemitricotyl, Type  $\beta$ . Transverse section near apex of hypocotyl.

FIG. 6. Hemitricotyl, Type  $\beta$ . Transverse section near base of hypocotyl.

$ph$  = phloem;  $px$  = protoxylem. All three figures show a considerable development of secondary xylem.

at the node than in the normal double bundle, the rotation still occurs at the usual level.

(b) The second group of hemitricotyls comprises two seedlings only, and in these the vascular strands of the abnormal cotyledon show considerable differences in structure and behaviour compared with those of the first

group. Each half of the bifurcated cotyledon exhibits a double-bundle midrib (Fig. 4), which gradually approaches its fellow until the adjacent phloems fuse (Fig. 7). This phase is followed by the reduction in size of the adjacent xylems and their fusion, so that a W-shaped structure, in

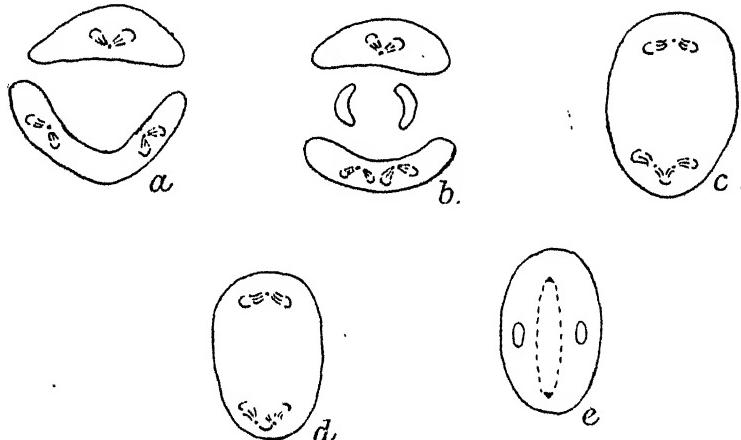


FIG. 7. *a-c.* Hemitricotyl, Type  $\beta$ . Diagrams showing the initiation of triarchy and its reduction to diarchy by fusion.

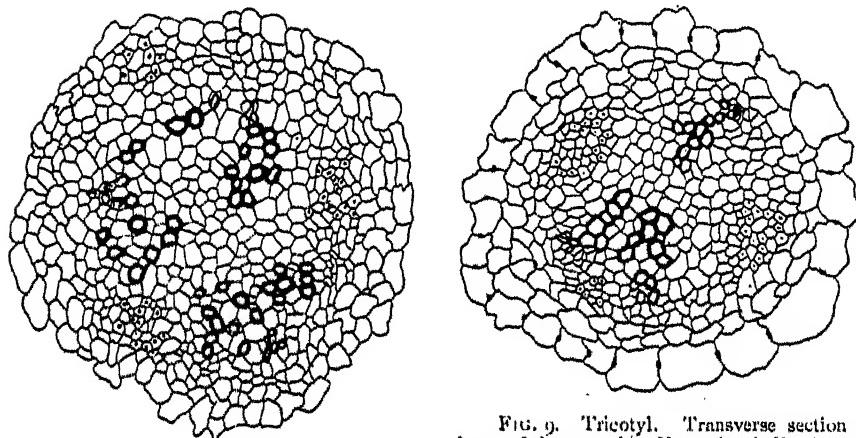


FIG. 8. Tricotyl. Transverse section near apex of hypocotyl.

FIG. 9. Tricotyl. Transverse section at base of hypocotyl. Note the indications of approaching fusion between two of the poles and the reduction in the size of the phloem group between them.

which the median portion is much flattened, results. This encloses a small phloem group, and is flanked laterally by two other phloem groups (Fig. 5). Ultimately, in one case at the base of the cotyledons and in the other in the hypocotyl, the fusion of these double bundles becomes more intimate, and the enclosed phloem strand dies out (Fig. 6). The two xylems also fuse as they pass down the hypocotyl, so that a diarch plate finally results. In both

the seedlings of this second group the cotyledon is split almost to the base. In the seedlings of the first group the epicotyledonary leaves, when present, are invariably two in number; but in one seedling of the second type there are three epicotyledonary leaves.

We propose to term those hemitrichocotyls which show single collateral bundles in the abnormal member 'Type  $\alpha$ ' and those which have double bundles 'Type  $\beta$ '.

#### TRICOTYLS.

The thirty-two tricotylous seedlings examined fall, with one exception, into a series which is readily derivable from the type  $\beta$  hemitrichocotyl. The earliest stage is one in which the triarch stage produced by the passage of the three double bundles into the hypocotyl is extremely transitory, one of the

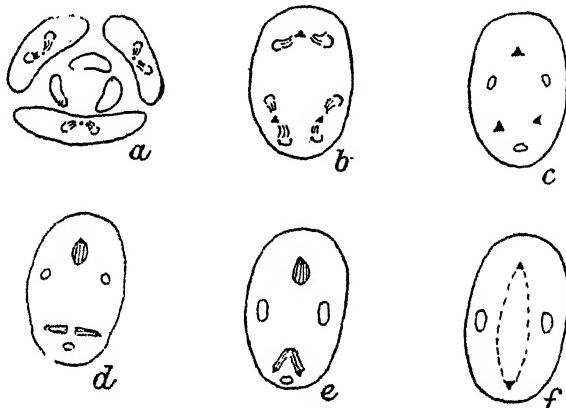


FIG. 10. a-f. Tricotyl showing reduction from triarchy to diarchy by fusion. Note the assumption of 'T' shape followed by 'Y' shape in the xylem during transition.

phloem groups becoming smaller and rapidly dying out, this being followed by the fusion of the xylem arms on either side of it. The fusion of the xylem arms to produce diarchy is in some cases preceded by the assumption of a 'T'-shape followed by a 'Y', in which the oblique arms are very short (Fig. 10); in other cases the 'Y'-shape is assumed directly without the intervention of a 'T' stage (Figs. 8 and 9).

From the type in which diarchy is rapidly developed, a series of stages is found in which diarchy is produced at a later and later stage, until it appears only in the apical region of the root, the hypocotyl and the major portion of the root showing a triarch structure. In other cases the apex of the root shows a 'Y' structure with two phloem groups, whilst others again show a distinctly unequal development of the xylem plate, two of the arms being less robust than the remaining one, and showing a relatively small phloem group between them. There is little doubt that if these seedlings had been allowed to develop further, the apical portion of the root would

have ultimately shown a diarch structure. Finally, there are those forms in which triarchy is established and persistent throughout; these, which include more than half the total number of tricotyls examined, forming the culmination of the series (Fig. 11).

One further point of interest must be noted. In one instance reduction from the triarch to the diarch condition was brought about not by the fusion of two xylem plates and the disappearance of the intervening phloem, but by the disappearance of a xylem plate, and the subsequent fusion of two phloem groups. The protoxylem was the first to disappear, followed later by the metaxylem. The possible significance of this interesting difference in the mode of reduction will be dealt with later.

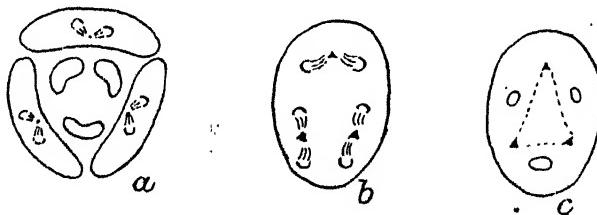


FIG. 11. a-c. Tricotyl showing persistent triarchy.

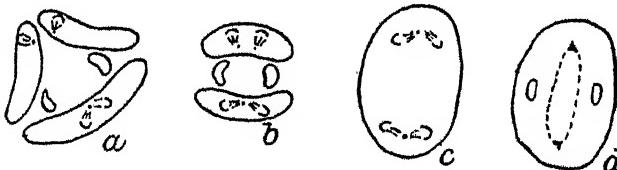


FIG. 12. a-d. Tricotyl, Type a.

In the tricotylous as in the hemitricotylous seedlings morphological characters gave no clue to the behaviour of the vascular strands. In some cases the cotyledons were of equal size, whilst in others two cotyledons were considerably smaller than the third; both conditions, however, being found to be associated with persistent triarchy. A feature which gave a much more reliable indication was found in the slightly earlier fusion of two of the cotyledons, as where this precocious union occurred it was usually followed by the ultimate union of the associated xylem poles.

The double bundles themselves showed a considerable variation in the degree of separation of the metaxylem elements of the triad, this being in some cases very pronounced, whilst in other cases the phloem only was double and the bundle appeared to have undergone lateral compression. No relationship could be traced, however, between the degree of compression and the ultimate fate of the vascular bundles.

Usually the young epicotyledonary leaves formed a trimerous whorl

alternating with the cotyledons, but in one case only two epicotyledonary leaves were present. It is interesting to note also that in some instances the vascular bundles of the epicotyledonary leaves showed the 'doubleness' which is so characteristic of the cotyledonary strands.

The exceptional case referred to at the beginning of this section is derivable from a type *a* hemitricotyl. In it two of the cotyledons possessed a collateral bundle, whilst the third showed a normal double bundle (Fig. 12). As they enter the hypocotyl the two collateral bundles are a little distance apart, but they commence to rotate and approach one another until the typical structure is produced, this occurring, however, at a rather lower level than in the case of the double bundle. Ultimately a diarch plate is formed. There were two epicotyledonary leaves present, these being situated on either side of the normal cotyledon.

#### HEMITETRACOTYL.

The single hemitetracotyl examined was essentially similar to the type *a* hemitricotyls, the two normal cotyledons having each a double bundle, whilst the midrib of each half of the bifurcated cotyledon was formed by a collateral strand. The two collateral strands behaved in transition as the constituent halves of a double bundle, so that triarchy was established immediately, and was maintained throughout the root.

#### TETRACOTYLS.

The twelve seedlings examined showed considerable variety in their vascular arrangements, but the majority formed a similar series to the tricotyls. In the simplest case the cotyledonary bundles, which are grouped in two obvious pairs, show a transient tetrarch arrangement at the top of the hypocotyl, this being followed by a reduction to diarchy without the intervention of a triarch stage, owing to the bundles fusing in pairs. This fusion is accompanied by the disappearance of the phloem group originally lying between each pair of xylem masses (Fig. 14).

The second type is one which leads up to stable tetrarchy and consists of the initiation of a tetrarch stage at the top of the hypocotyl, followed sooner or later by a reduction to the triarch condition (Fig. 15). In some seedlings the tetrarch stage was very brief, but in others it was retained farther and farther down the hypocotyl, until finally the tetrarch arrangement obtained throughout (Fig. 16).

The reduction from tetrarchy to triarchy is accomplished in two different ways, the two methods being almost equally represented among the seedlings. In some instances reduction is brought about by the fusion of two of the xylem masses, this being preceded by the loss of the phloem lying between them (Fig. 15). In the second type of seedling the reduction is produced by the gradual dying out of one xylem plate and the fusion of

the phloem groups on either side of it. In almost every example of this type the protoxylem disappears first, followed at a later stage by the

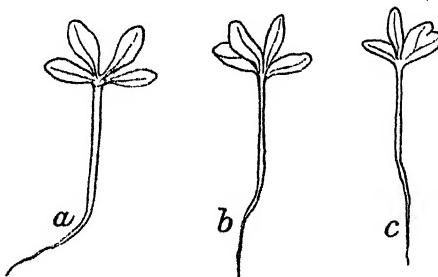


FIG. 13. *a, b, c.* Tetracotylous and hemitetracotylous seedlings.

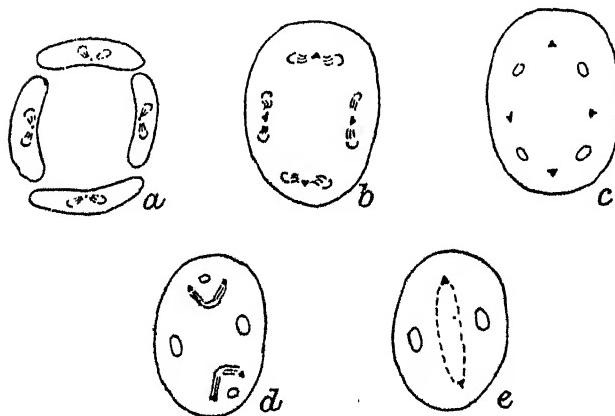


FIG. 14. *a-e.* Tetracotyl showing reduction from tetracytary to diacytary by fusion.

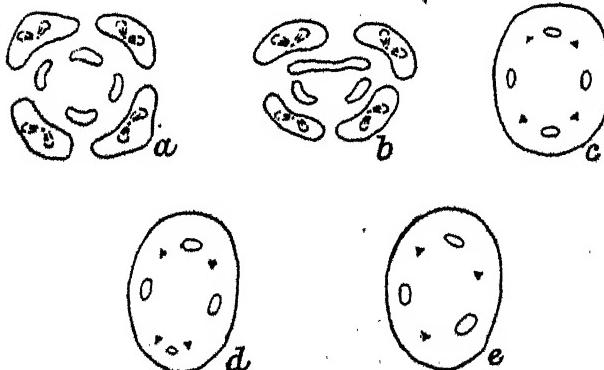


FIG. 15. *a-e.* Tetracotyl showing reduction from tetracytary to triacytary by fusion.

metaxylem (cf. Compton [4], *Cannabis sativa*, Tricotyl D). In one case only does the metaxylem disappear, first leaving an isolated protoxylem

which persists until a later stage, thus resembling the condition of affairs characterizing *Phacelia tanacetifolia* as reported by Compton (4).

In one case a seedling, which had shown reduction from tetrarchy to triarchy by the disappearance of a xylem plate, showed a further reduction to diarchy in the root, this being accomplished by the fusion of the xylem masses and the obliteration of the phloem group lying between them (Fig. 17).

One other type remains to be described in which two of the cotyledons had double bundles, whilst the other two showed collateral bundles which in the transition region behaved as the constituent halves of a double

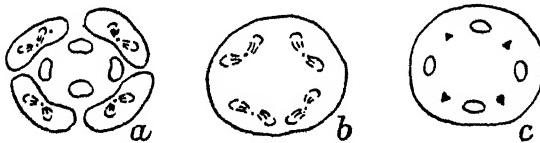


FIG. 16. a-c. Tetracyl showing persistent tetrarchy.

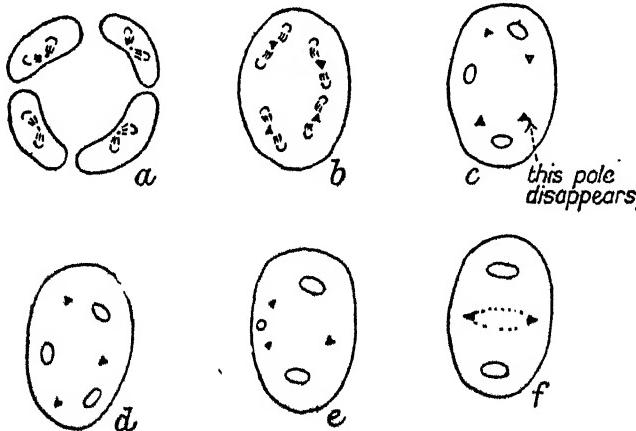


FIG. 17. a-f. Tetracyl showing reduction from tetrarchy to triarchy by loss of one xylem pole, and from triarchy to diarchy at a later stage by fusion.

bundle. Triarchy is thus immediately established and persists throughout the seedling, although there are signs of incipient diarchy near the apex of the root, one phloem being very much reduced, whilst the poles on either side are quite near to one another.

The epicotyledonary leaves appear to be three in number in most cases, but in one there are four leaves, and a further example shows the interesting condition in which two normal leaves and one exhibiting partial fission are evident (Fig. 15, a, b).

It will thus be perceived that in the assumption of tetrarch symmetry there is a distinct tendency on the part of the first epicotyledonary leaves to

lag behind the members of the cotyledonary whorl, a feature which was evident to some extent in the tricotylous and hemitricotylous series, and which has already been noted by Compton (4) in *Cannabis sativa*.

#### DISCUSSION.

Although the references to polycotylous seedlings are relatively numerous in botanical literature, they are as a rule merely incidental, and in the majority of cases the anatomy of such seedlings does not seem to have been investigated. This is especially the case with the earlier papers, which are little more than records with brief anatomical notes in one or two instances.

Léger (14) has dealt somewhat fully, however, with the abnormal seedlings which are frequently found in *Acer platanoides*, and Dangeard (6) in his investigations on the anatomy of coniferous seedlings has also attempted a classification of the seed leaves met with in the polycotylous genera of that group.

The series of papers by Hill and de Fraine on the anatomy of gymnospermous seedlings (9) is, however, the first in which a systematic attempt is made to show how the polycotylous condition may have arisen; and although this occupies a subordinate position in the general scheme of their investigation, they elaborate a theory of whole cotyledons, half cotyledons, and subsidiary cotyledons, the basis of which is the part taken by the cotyledonary strand in root formation. Thus *whole* cotyledons are distinguished by the fact that the vascular bundle of the cotyledon forms a root-pole quite independently; *half* cotyledons, on the contrary, being characterized by their bundles uniting in pairs, each pair forming a root-pole; whilst the vascular bundles of the *subsidiary* cotyledons merely fuse on to the other bundles without taking any direct part in root formation. This theory they support by a considerable body of evidence, and they interpret certain cases as illustrating the 'promotion' of subsidiary and half cotyledons to higher rank as whole cotyledons.

Compton (4), in that section of his paper dealing with schizocotyly, criticizes certain details of this scheme, taking exception to the class of subsidiary cotyledons, and to the idea of 'promotion', and to these criticisms Hill and de Fraine (13) have replied in a further paper.

In addition to the papers of Hill and de Fraine, and Compton, a number of isolated references to the anatomy of polycotylous seedlings are to be found in the majority of the more recent contributions to seedling anatomy, and these will be referred to when necessary.

With regard to the seedling anatomy of the wallflower itself, as mentioned above, that of the normal plant has been described by Thomas (20) and also by Scott (17). In addition Thomas (20) mentions tricotylous specimens of *Cheiranthus Cheiri* and of *Sisymbrium carpaticum*, both showing persistent triarchy, and one of *Matthiola tricuspidata* in which a

diarch root is produced by the early fusion of two of the double bundles, thus producing a further illustration of a transition stage from a type  $\beta$  hemitrichotyl. On the basis of our work we propose to put forward the following provisional explanation of the origin of the polycotylous condition. Polycotyly may arise in one of three ways, namely, either by cotyledonary fission, by the division of the growing point of the cotyledon, or by the displacement of one of the foliage leaves into the cotyledonary whorl. The first type of increase, by fission, we consider to be illustrated by our type  $a$ , in which each of the half cotyledons possesses a midrib of the collateral type. This we regard as derived from a double bundle by the wide separation of the constituent halves, with which has gone a loss of the median protoxylem. The protoxylem is indeed extremely transient in character even in the normal cotyledon, and it may well be that it is occasionally ontogenetically obsolete.

It will be noted that this method of cotyledonary increase owes its origin to the *qualitative* division of the parent cotyledon, and although the fission may, though rarely, occur so early that the tricotylous condition obtains, yet the bundles invariably behave as the constituent halves of a double bundle, giving rise to one pole of the root.

This type of cotylar increase is extremely common, and a consideration of Hill and de Fraine's work will show that the majority of their half cotyledons<sup>1</sup> belong to the same category. It is notable that in two of the seedlings described by them, namely, *Pinus contorta* var. *Murrayana*, series A (cotyledons C and D), and *P. sylvestris*, series A (cotyledons  $d$  and  $e$ ), their origin is shown by the fact that the median resin duct passes to one member of the pair and assumes in the hypocotyl the characteristic position found in the whole cotyledon. In our opinion, however, cotylar fission of the qualitative type constitutes a finite series, and has led to no further developments.

Cotyledonary increase produced by the early division of the cotylar apex—our type  $\beta$ —is essentially different, being *quantitative* in character, and thus contrasting strongly with the division of type  $a$ . It is evident that this type of increase, which is illustrated by a perfectly graded series in our material, would offer an ideal method for the development of the polycotylous condition without calling in the aid of a system of 'promotions' which, to say the least, involves considerable morphological changes.

The initial member of such a series would be a type  $\beta$  hemitrichotyl in which the normal structure is established either in the basal portion of the

<sup>1</sup> e.g. *Cupressus torulosa*, series C and D; *C. macrocarpa*, series B; *Abies Balsamea*, series A; *A. pectinata*, series A (and the six half cotyledons of series B, C, and D); *A. magnifica* var. *shastensis*; *Picea ajanensis*, series A, B, C, and D; *Cedrus atlantica*, series A, B, and C; *C. deodara*; *Pinus Pinea*, series A, B, and C; *P. gerardiana*, *P. canariensis*, *P. contorta* var. *Murrayana*, series A (half cotyledons C and D); and *P. sylvestris*, series A (half cotyledons  $d$  and  $e$ ).

bifurcated cotyledon or at the apex of the hypocotyl. This would be succeeded by tricotyly with the assumption of diarchy at the apex of the hypocotyl, the subsequent stages being provided by a progressively longer delay in the fusion of the vascular elements concerned until ultimately persistent triarchy is attained. Granted a similar shifting to an earlier stage of the apical quantitative division in phylogeny, the transition from dicotyly to polycotyly would be relatively simple. It will be obvious, moreover, that from such an initial stage a large range of possible developments is conceivable, since the process is capable of infinite repetition and may be augmented by a qualitative fission such as is illustrated by our type *a* cotyledons.

A consideration of previous investigations will demonstrate that this type of cotyledonary bifurcation is of fairly frequent occurrence. Thus Lee (15) records an instance in *Dimorphotheca pluvialis* and in *Bidens pilosa*, and Guillaumin (8) in *Schinus terebinthifolius*. The seedlings of *Acer platanoides* described by Léger (14) all seem to fall within this category, though the elaboration of the lateral bundle systems produces further complications, whilst of Compton's material (4), hemitricotyls A and B, hemitricotyls C and F, and all the tricotyls of *Cannabis sativa*, tricotyls D and E of *Lepidium sativum*, and the single hemitricotyl of *Ulex europeus* conform exactly.

With regard to Hill and de Fraine's Gymnosperm material it will be perceived that the four half cotyledons in series A, and the two half cotyledons in series B, *Pinus australis*, the four half cotyledons (C and D, G and H) of *Pinus insignis*, series B, the two half cotyledons of *P. contorta* var. *Murrayana*, series I, the two half cotyledons of *P. montana* var. *gallica*, series C, and the two half cotyledons (*f* and *g*) of *P. sylvestris*, series H, are also essentially similar. It is of extreme interest to note the behaviour of the median resin duct in the only two members of the above series figured by Hill and de Fraine, namely *P. australis*, series A, and *P. sylvestris*, series H, and contrast it with what obtains in those to which we have previously referred, as illustrating qualitative as distinct from quantitative cotylar division. Here it will be seen that each half cotyledon possesses a median resin duct flanked by the halves of a normal 'double bundle'. The resin ducts fuse at the apex of the hypocotyl, whilst the behaviour of the xylem and phloem resembles that described by us for similar cases in *Cheiranthus*.

One further instance from this important series of papers calls for comment, namely, that of *Araucaria Cunninghamii*, series A and B. It will be noted that Hill and de Fraine demonstrated that the appearance of tetracotyly is deceptive and that there are really only two deeply bifid cotyledons. The half cotyledons are each traversed by two collateral bundles which partially fuse near the apex of the hypocotyl. The four bundles thus produced pursue a completely independent course throughout the major

portion of the hypocotyl, each ultimately fusing with its fellow to form the two poles of a diarch root. It will be perceived that these seedlings afford an admirable illustration of an advanced stage in the evolution of polycotyly along the lines we have indicated as probable.

In addition to these two methods by which the plant may become polycotyledonous, another possibility presents itself. Several investigators of seedling anatomy have reported the 'doubleness' of the vascular bundles of the early epicotyledonary leaves in some species.

Thus Davey (5) reports this in *Juglans* spp., *Fagus sylvatica*, and *Castanea sativa*, while it is described by Thomas (20) in *Cheiranthus maritimus* and *Draba Aisoon*. It has also been observed by us in some of the *Cheiranthus* seedlings examined during this investigation. In *Juglans nigra* the double bundles from the first two epicotyledonary leaves form, quite independently, two of the poles of the tetrarch root, and the same phenomenon is reported by Compton (3) in *Caesalpinia sepiaria* and in *Pithecellobium Unguis-cati*. Bearing in mind these facts and also that Hill and de Fraine (9) interpret some of the phenomena in the polycotylous gymnospermous seedlings as indicating that a plumular leaf has been displaced to the cotyledonary whorl, it seems quite possible that the cotyledon number may be increased by the precocious development and displacement of one of the epicotyledonary leaves, the double bundle of which shares directly in root formation. It is evident from these that the 'promotion' of a displaced epicotyledonary leaf to the rank of a whole cotyledon would not necessarily involve an intermediate phase as half cotyledon such as Hill and de Fraine suggest in their system of evolution. There is, however, no direct evidence of the displacement of an epicotyledonary leaf in our material. It has been pointed out previously that there are two methods of reduction of the root pole number, namely by fusion of poles, and by the loss of one xylem pole. This second method is described also by Compton (4) in *Cannabis sativa*, tricotyl D, and in the majority of the abnormal seedlings of *Phacelia tanacetifolia*. The bundle which shows this behaviour is regarded by Compton as belonging to a 'subsidiary' cotyledon, but with this interpretation we cannot agree, if the term 'subsidiary' is to be applied in the sense used by Hill and de Fraine. It is conceivable that the two methods of reduction are connected with different methods of cotylar increase, reduction by fusion occurring when polycotyly is produced by the division of the apex of the cotyledon, and reduction by disappearance appearing in seedlings possessing a displaced epicotyledonary leaf. The occurrence, however, of intermediate stages between fusion and suppression militates strongly against this interpretation of the method of reduction under consideration.

Thus in one or two *Cheiranthus* seedlings which showed reduction from triarchy to diarchy by fusion of xylem poles, one of the two poles became somewhat smaller than the other, just prior to fusion, thus presenting an

appearance intermediate between ordinary fusion and reduction by the dying away of a xylem mass, and this intermediate condition has been noted in a more pronounced degree in some tricotylous seedlings of an undetermined species of *Matthiola*. Two of these show a loss of protoxylem in one of the two xylem plates which ultimately merge, whilst a third shows a loss of metaxylem, so that before fusion one xylem plate is represented simply by a separate strand of crushed protoxylem elements.

It was thought possible at first that the reduction by fusion and disappearance might be successive stages in the acquirement of persistent triarchy. If this were true, however, one would expect the dying away of a pole to occur always low down in the root, since it would constitute the stage immediately preceding complete and persistent triarchy. This is, however, not the case, for in some instances among the tetracotylous seedlings the tetrarch stage is reduced to a triarch one in the hypocotyl by the dying away of a xylem plate, whilst reduction by fusion of poles is found in the root.

All things considered it seems preferable to regard this type of reduction in the number of poles as either a subsidiary line of evolution or as due to a sudden dominance of the ancestral hypocotyledonary diarchy over the newly acquired triarch symmetry.

It will be obvious, however, that though there is a considerable body of evidence which lends support to our hypothesis concerning the origin of polycotyly, there are at the same time a number of difficulties which present themselves. In the first place, it may be suggested that there are structures which are obviously half bundles of a cotyledon which each show double structure, as for example in the Calycanthaceae, Fagaceae, Euphorbiaceae, Sapotaceae, Ebenaceae, and certain of the Rosaceae, in which the so-called diagonal arrangement occurs. A careful consideration of these will show an essential difference however, since in the majority of cases a very evident median protoxylem exists *between* the paired double bundles, and even in the extreme case of *Calycanthus* Chauveaud has demonstrated the existence of such a strand in the very young state. No trace of a median protoxylem has ever been found by us between the twin double bundles in the *Cheiranthus* seedlings, and such might reasonably have been expected had they originated from modified half bundles. It must be admitted, however, that no median protoxylem appears in the separate parts of the split cotyledons between the two collateral bundles of the type  $\alpha$  seedling, which are recognized as half bundles. There is, however, a further important distinction, for, in the groups named above, the constituent halves invariably constitute a *divergent* system, and in no case do they unite at a lower level, while in the type  $\beta$  *Cheiranthus* seedlings the bundles form a *convergent* system, and in many cases unite either in the hypocotyl or in the root.

Another difficulty is found, not so much in *Cheiranthus* itself as in the application of the theory to seedlings of other genera. Thus Hill and de Fraine (10) describe a seedling of *Silene Schafra* which, although dicotyloous, is yet triarch throughout, the larger of the two cotyledons possessing two double bundles. The same feature, though not in so pronounced a form, has been met with by one of us in polycotyloous seedlings of *Centranthus rubra*. These seedlings are obviously of type  $\beta$ , and the structure of the cotyledons may possibly be due to a fusion or partial fusion of the parenchymatous tissues of the lamina having followed the division of the cotylar apex. Such a fusion is quite probable when one considers the close proximity of the two cotyledons during their development, and the plastic character of the tissues during the early stages. A second possible explanation is that whilst the plerome and the outer half of the periblem and dermatogen have retained their full activity subsequent to apical division, the activity of the inner half of the periblem and dermatogen has been completely suppressed, since a less complete reduction in the activity of these tissues is often apparent in cotyledons which show incomplete division.

A difficulty of another kind arises in connexion with *Lotus corniculatus* and *Carmichaelia australis*, two Leguminosae described by Compton (3).

*Lotus corniculatus* is of considerable interest, since the dicotyl exhibits a trimerous symmetry due to the persistence of one of the lateral strands from each cotyledon in the hypocotyl, these fusing to form a third pole; and a similar state of affairs is found in *Carmichaelia australis*. In the former species Compton describes a hemitricotyl in which root and hypocotyl showed triarch symmetry, but which obviously corresponds to our type  $\beta$ . The tricotyloous specimens of *Lotus corniculatus* and *Carmichaelia* also show a trimerous symmetry; but we are inclined to regard this as not homologous with that of the dicotyl and hemitricotyl, but rather as an instance of the complete suppression of the relatively feeble pole of the triarch system derived from the fused laterals, and its replacement by the robust median strand derived from the midrib of the third cotyledon.

This view is supported by an examination of the normal seedling structure of the genus *Lotus*, and in fact of the whole tribe Loteae. In *Lotus* the cotyledons each contain a median double bundle and two laterals, from which in *L. tetragonolobus* and *L. Requienii* tetrarchy results, while in *L. corniculatus* triarchy is characteristic of the root. This is evidently due to the suppression of one of the 'paired laterals', and it is noteworthy that the other is in process of suppression. Other members of the tribe Loteae show similar features, e.g. *Anthyllis tetraphylla* is tetrarch, while *A. vulneraria* and *A. Barba-Jovis* are diarch, and another member, *Dorycnium hirsutum*, may be either tetrarch, triarch, or diarch [(3) pp. 35-9]. A similar tendency to the suppression of laterals is also found in tribe Galegeae,

sub-tribe Robiniinae [(3) pp. 41-5], to which *Carmichaelia australis* belongs. In view of these facts it seems reasonable to suppose that the robust strand of the new cotyledon would supplant the feeble pole derived from the laterals.

It has not been felt to be necessary to deal with the class of cotyledons termed 'subsidiary' by Hill and de Fraine since, they have no bearing on our work. This group is left in rather an ill-defined condition, and seems to contain all cases which cannot be definitely classed as 'whole' or half cotyledons. Although they do not make any definite statement it is evident that they include in this class at least two types of cotyledon :

(a) displaced foliage leaves ;

(b) cotyledons formed by the splitting off of tissues laterally from the normal cotyledon, the splitting being either symmetrical or asymmetrical.

The variety of behaviour, however, is so great, and the number of seedlings of each species examined is relatively so small, that on the present evidence it seems impossible to put forward any comprehensive explanation of the phenomena found in these seedlings. A more thorough investigation of two or three species seems to be called for, and such an investigation is now in progress.

#### SUMMARY.

1. The vascular anatomy of a series of wallflower seedlings showing cotyledonary abnormality ranging from hemitricotyl to tetracotyl is described.

2. This is believed by us to indicate at least two, and possibly three, methods of cotyledonary increase :

(1) By cotyledonary fission ;

(2) By dichotomy of the growing point of the cotyledon ; and

(3) Much more doubtfully, by the downward displacement of one or more epicotyledonary leaves.

3. It is finally shown that previous work on schizocotyl is capable of interpretation on this basis, and affords illustrations of all these types of increase.

The authors are much indebted to Dr. T. G. Hill for his courteous permission to examine a number of slides of Gymnosperm seedlings, and also to Professor Carr, University College, Nottingham, who granted every facility for the investigation.

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# Absorption of Gold from Colloidal Solutions by Fungi.

BY

MAUD WILLIAMS, B.Sc.

IT has been known for some time that colloidal preparations of gold produced by the addition of tannic acid tend to become mouldy, and that the mould, in course of time, removes the metal from the solution (1).

Upon the suggestion of Mr. E. Hatschek an attempt was therefore made to find:

- (a) if the same result could be obtained with solutions prepared by Zsigmondy's method (2) (addition of formaldehyde and sodium carbonate).
- (b) what fungus or fungi could bring about the change.
- (c) in what region the gold was retained.

## PREPARATIONS.

A series of solutions was prepared by each method, the colours of the solutions ranging from red to blue, according to the sizes of the gold particles.

To solutions prepared by Zsigmondy's method, gum arabic was added to serve as a nutrient.

Spores of *Penicillium glaucum*, *Aspergillus niger*, and *Oidium lactis* were introduced into separate flasks of the solutions containing about 150 c.c. of liquid. Cotton-wool plugs were inserted and the flasks kept in darkness at room temperature for upwards of fifteen months.

As hyphae developed the material was examined microscopically, and finally, when considerable dimensions had been attained, the fungus was treated with chromacetic fixative or absolute alcohol, embedded in wax, and microtomed.

## OBSERVATIONS.

About one week after the spores were introduced into the flasks, hyphae had appeared in the cases of all three fungi.

*Aspergillus niger* showed no coloration due to the solution, and quickly died.

*Penicillium* and *Oidium* both showed faint red, purple, or blue in the hyphae, according to the colour of the solution used.

From this time onwards growth of the fungus was always much quicker in the tannin preparations than in the Zsigmondy solution.

Oval or circular masses formed, sometimes on the surface, sometimes entirely submerged. Deeply tinted central regions developed with paler peripheral parts. As the gold accumulated in a region the colour gradually became blue. Material had to be cultivated for periods of four to nine months before the patches were sufficiently large to be embedded. For each method of preparation it was noticed that colour appeared in the hyphae more rapidly in the case of red solutions than in the case of blue. This point is of interest since it is known that the red solutions contain smaller particles (3), and diffuse more rapidly than the blue (4).

After material had been kept for ten to twelve months it was common to find the conidial stage again reached. Great development of intercalary spores was characteristic of most of the *Oidium lactis* cultures.

In no case was precipitation of the gold in the flasks observed, and there was a tendency for the liquids themselves to become alkaline. Thus six specimens prepared in December, 1915, and tested in October, 1916, all showed action on red litmus paper, although they had been prepared by means of tannic acid.

#### CONTROLS.

1. To find whether any of the observed colouring was due to the influence of tannic acid or gum arabic, material was grown upon these substances.

2. The alcohol, xylol, and oil of cloves used in the microscopic preparations were each added to separate portions of the solutions. No precipitation was produced.

The chromacetic fixative, when added in large amounts to specimens of the solution, produced a brownish precipitate, but this was quite unlike any colouring observed in the preparations.

3. Deeply coloured patches of fungi were soaked in chlorine water, with the result that the gold could be completely removed.

4. Cultures of the fungi used were grown upon tannic acid and gum arabic, then killed by soaking in absolute alcohol, well washed and added to solutions in order to find whether retention of the gold would result.

#### EXAMINATION OF SECTIONS.

A striking feature of all sections was the small amount of protoplasm in the hyphae. In the alcohol preparations the plasmolysis of the protoplasm was helpful in determining the region of retention as the specimens

were of extremely small diameter, the *Oidium hyphae* being the easier to examine.

The results were as follows :

#### 1. ABSORPTION BY LIVING MATERIAL.

##### *Oidium lactis.*

- (a) Gold present throughout the wall, as seen in cross-section.
- (b) No gold retained in the protoplasm.
- (c) Wall of spore coloured in same manner as wall of hyphae.

##### *Penicillium glaucum.*

- Wall of hyphae stained throughout its thickness.
- Protoplasm free from gold.
- Wall of spore (which is cuticularized) without stain or surface deposit.

#### 2. ABSORPTION BY DEAD FUNGUS.

Some coloration seen in wall of hypha.	Same result as <i>O. lactis</i> .
Distribution less uniform than in case of living material.	" "
Slight precipitation of gold on limited areas of surface of protoplasm.	" "

In all the specimens examined the gold was firmly retained and could not be removed during the embedding, or by long washing in such liquids as ether, acetone, and alcohol, of very varied surface tensions.

#### METHOD OF REMOVAL AND RETENTION OF THE GOLD.

Since the colloidal gold carries a negative charge and can be precipitated by the addition of electrolytes, two means by which the fungus acts suggest themselves :

(a) The fungus wall may possess a positive charge when in contact with the colloidal solution.

(b) Electrolytes may diffuse out of the cell at such a rate that they only suffice to coagulate the gold which has diffused into the wall and not so as to affect the liquid outside. Experiments were accordingly made to endeavour to find the nature of the charges upon the fungus cellulose in contact with water and with gold solutions.

A microscopic cover-slip was provided with electrodes of silver foil fixed in position with shellac and carrying fine wires. The material to be examined was placed on the slide and the whole connected with a reversing key and battery.

In some cases, when the current from four Leclanché cells was used, a well-marked migration of tiny pieces of hyphae and of spores took place, this movement being immediately reversed upon reversing the current.

The direction of migration, and consequently the charges, were varied ; thus of twelve cultures examined, six showed a negative charge, three no charge, while three showed a slight positive charge.

The first suggestion of an electrical explanation was therefore not supported.

The only evidence at present in support of the second suggestion is the final alkalinity of the tannic acid preparations after all the gold has been removed by the fungus.

#### SUMMARY.

1. Conidia of *Penicillium glaucum* and *Oidium lactis* can develop in colloidal gold solutions which contain tannin or gum arabic.
2. The living fungus, during growth, removes the metal from the solution. Retention occurs in such walls as are not cuticularized.
3. The process goes on more irregularly when masses of dead fungus are introduced into solutions.
4. Solutions with a higher rate of diffusion colour the fungus more quickly than those with a lower rate.

In all cases the accumulation of gold finally produces a blue coloration.

5. No explanation of the process can be given.

In conclusion the writer wishes to express her best thanks to Mr. Hatschek and Dr. Willows for many suggestions with regard to the physical preparations and determinations, and to Mr. H. B. Lacey for much help in the botanical matters.

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# Sphagna, their Habitats, Adaptations, and Associates.<sup>1</sup>

BY

W. WATSON, D. Sc.

With five Figures in the Text.

## INTRODUCTION.

THE familiar names of bog-moss, turf-moss, and peat-moss indicate the general habitats of the different species of *Sphagnum*, since they are all found in places where peat is present, and in most cases where the ground is wet and has that yielding character which is associated with the word boggy. The degree of bogginess varies greatly; in some cases the *Sphagnum* layer conceals a treacherous depth, in others a firm substratum is quickly reached. In exceptional cases peat is absent or almost so, but if not present in the particular spot where the *Sphagnum* actually grows can be found in the near vicinity. *Sphagnum inundatum*, *S. subnitens*, *S. recurvum*, and other species are sometimes found on the sides of furrows cut by streamlets, or in other spots where the immediate presence of peat is not apparent, but usually little search is necessary to establish the presence of peat in the neighbourhood. One of the most striking cases of this kind noticed by the author occurs by the side of a sloping hard-metalled road on the Blackdown Hills in Somersetshire. *S. pulchrum* is present in fair quantity and seems to obtain its supply of water from the inconstant drainage after rain; the district, however, is turf-y and further search reveals the presence of patches of *S. recurvum* which are constantly moist and have a somewhat peaty substratum. Sphagna sometimes occur on moist rock-ledges which have little or no peaty covering. This very rarely happens in lowland or even in upland districts, but is not uncommon on sheltered alpine or subalpine ledges, where the moisture from the clouds forms no inconsiderable part of the water-supply. As concrete instances of this habitat noticed by the author the following may be given: *S. acutifolium* on moist rock-ledges, altitude 3,500 feet, Ben Doran, Argyllshire; *S. molluscum*, with a similar habitat on the same mountain, but at a lower elevation (2,500 feet) and in company with *Scapania ornithopodioides*.

When the water is squeezed out of these tufts of *Sphagnum* it is found to be more or less acid; the peat derived from its decay seems to retain this

<sup>1</sup> This paper forms part of a thesis for the degree of Doctor of Science, London.

acidic character, and when the catchment area for an urban water-supply is coincident, special means have to be adopted to prevent the so-called 'humic acids' from attacking the lead pipes. Much research work has been done on this acidity of *Sphagnum* and a great deal of literature deals with it.<sup>1</sup> This acidic nature of Sphagna has a great effect on their habitats and distribution, and the main purpose of this article is to attempt to correlate their morphological with their ecological characters.

The distribution in regard to altitude is more affected by human agency than through any discrimination by the species themselves. Many of our low-lying 'mosses' have been drained, the reclaimer has extended

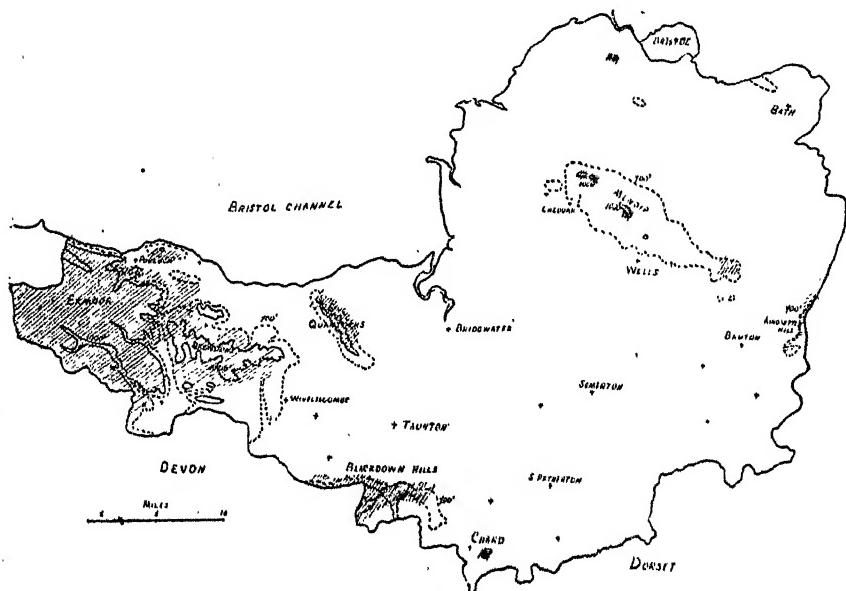


FIG. 1. Sketch-map of Somerset to show the distribution of Sphagna.

The areas where Sphagna have been found are shaded; dotted lines represent the contour-line of 700 feet, the continuous lines indicate the contour-line of 1,000 feet. A few towns are indicated by crosses.

the land available for agricultural purposes, and in England Sphagneta are seldom found below the altitude where it is economically possible for the farmer to bring the land into cultivation. This altitude varies according to many other influencing factors, but generally is about 700 feet. Some of our lowland Sphagneta which still exist owe their survival to the excellent cover for game which is afforded by associated plants such as *Betula*, *Myrica*, and *Molinia*. The accompanying map of Somerset (Fig. 1) shows the altitudinal distribution of *Sphagnum* and illustrates how it is seldom

<sup>1</sup> See Skene: The Acidity of *Sphagnum* and its Relation to Chalk and Mineral Salts. Ann. of Bot., January, 1915.

found below 700 feet in this agricultural county. The only places below 700 feet where *Sphagna* are found are on the flanks of the hills, a *Myrica* heath near Chard, an Old Red Sandstone area near Bristol, and isolated areas on the peat-moor of Central Somerset. The *Sphagnum* occurring at the lowest elevation (50 feet) is *S. subnitens* (= *S. plumulosum*), and this species is also present near the summit of Dunkery Beacon, the highest (1,700 feet) hill in Somerset. The distribution of the different species growing in the county has also been worked out, but the altitudinal range is too limited and human interference has been too prominent for any useful result to be attained by more detailed and specific mapping of the data available. The map, however, clearly shows the preference of *Sphagnum* for non-calcareous areas. In the hilly Mendip region of East Somerset it only grows in a few isolated areas where inliers of Old Red Sandstone occur, whilst on the surrounding Carboniferous Limestone it is entirely absent. The central peat-moor (Sedgemoor) is chiefly on a substratum of Jurassic calcareous beds with alluvial clays and a few recent marine beds, and it is only in the uncultivated areas where the calcareous character is neutralized that *Sphagnum* occurs, and such patches are isolated and rare. All the other *Sphagnum* regions are underlain by siliceous and argillaceous rocks, especially those of the Devonian and Old Red Sandstone system. The geological distribution of Somerset Sphagneta may be summarized as follows:

Devonian:—The hilly region to the west of the county, including Exmoor, the Brendons, and the Quantocks.

Old Red Sandstone:—Isolated areas on the Mendip hills and a small patch west of Bristol.

Lower and Mid-Lias argillaceous beds:—Small areas on Chard Common and on the Blackdown hills.

Kimeridge and Oxford Clays of the Oolite:—Small and isolated areas on the eastern boundary.

Greensand:—Portions of the Blackdown hills.

Detailed maps of some uncultivated or semi-cultivated portion of Scotland would probably lead to good results as regards the range in altitude of the different species, but at present sufficient data for such maps are not available, though there is some evidence that species such as *S. acutifolium*, *S. fuscum*, *S. rubellum*, *S. Lindbergii*, *S. Girgensohnii*, and *S. papillosum* should be considered as plants of elevated moorlands, whilst such species as *S. fimbriatum* and *S. Warnstorffii* are normally plants of low elevations. In Somerset *S. rubellum* and *S. papillosum* occur at an altitude of 320 feet, and *S. Girgensohnii* is found at 600 feet, and similar (and even more striking) exceptions are known in other counties.

Little definite information can be given as to the specific endurance of sub-halophytic conditions. None of the *Sphagna* appears to have any tolerance of such habitats; they very rarely occur on sand-dunes, and abhor

salt-marsh associations. *S. subnitens* grows sparingly on the dunes near Southport; *S. imbricatum* is said to prefer wet heaths and bogs near the coast, but the author has found it more common in inland regions, in one case preserved in peat cuttings on the Pennines in a district where the living plant is now extinct. Experimental work with culture solutions has shown that the effect of high concentration of salts is variable according to the salt and the species employed, and that a concentration useful to most plants generally acts injuriously.

#### PROTECTIVE DEVICES.

*Sphagnum* is often regarded as a pronounced hydrophyte, but any bryologist who carefully studies its structure and compares it with that of other Mosses must be struck by the presence of a number of characters which in other plants would be considered as of a xerophytic nature, enabling the plant to endure periods of drought. Such characters as compactness of habit, imbrication of the leaves, concave leaves with hooded or inrolled apical portions, formation of capillary chambers along which water passes, papillosity of the cell-membrane, intermixture of dead empty cells with living chlorophyllous ones, and presence of reservoirs for the storage of water, are usually accounted xerophytic devices in other Mosses, and all these characters are shown by species of *Sphagnum*.

Imbrication of the branch-leaves to a greater or less extent is of general occurrence in the Sphagna, being entirely absent only in species which are usually found submerged (e. g. *S. cuspidatum*, Fig. 4, A) or in shaded moist situations (e. g. *S. squarrosum*). In some plants of drier moorlands, e. g. *S. compactum* (Fig. 5, W), *S. cymbifolium* var. *congestum*, *S. papillosum* var. *confertum*, the imbrication is very pronounced; in fact it is a general rule that the drier the habitat the closer is the imbrication. *S. subnitens* (Fig. 2, A) in other respects has a similar structure to *S. quinquefarium*, but the latter usually grows in drier places, and has its leaves more closely imbricated.

The branch-leaves of nearly all species are more or less concave, but generally the most pronounced hydrophytic member of a group of allied species has the leaves least concave. In the Cuspidata group, *S. cuspidatum* has a much flatter leaf (Fig. 4, B) and a wetter habitat than *S. pulchrum* (Fig. 4, F); similarly in the Subsecunda section, *S. obesum* (Fig. 5, R) may be compared with *S. subsecundum* (Fig. 3, K). The leaves, it may be noted, are usually larger or longer in the more aquatic species.

Inrolling of the branch-leaf is another character which is general for the Sphagna. The lateral margins of the upper half of the leaf become incurved so that two small chambers are formed (Fig. 2, G), or the incurving may be so pronounced that a tube results (Fig. 4, F). In some Sphagna the inrolling is little shown, but the leaf is very concave and is cucullate, i. e. its

apex is curved over so as to form a hood (Fig. 5, I). This cucullate leaf is a distinctive character of the Cymbifolia section (*S. cymbifolium*, *S. papillosum*, *S. medium*, *S. imbricatum*), but is slightly shown in members of other groups, e.g. *S. rubellum* (Fig. 3, E).

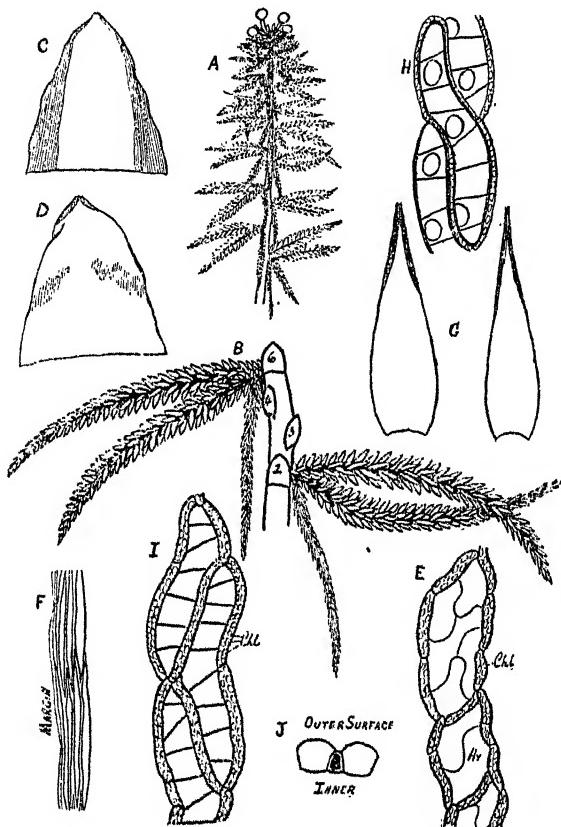


FIG. 2. *Sphagnum subnitens*, R. and W. A. Diagrammatic sketch of the fertile plant,  $\times \frac{1}{2}$ . B. Portion of the plant showing two fascicles or groups of branches,  $\times 3$ ; the stem-leaves are numbered to show the arrangement, leaves 2 and 5 being on the other side of the stem. The leaves 3 and 4 are usually nearer together than they are shown in the figure. C. Stem-leaf,  $\times 17$ , indicating the border of narrow cells becoming broader below. D. Stem-leaf,  $\times 17$ , showing undulations and the margin involuted at apex. E. Cells from the middle of the stem-leaf,  $\times 210$ . Chl. Chlorophyllous cell with chlorophyll granules. Hy. Hyaline or empty cell which is usually divided by oblique septa. F. Cells from the margin of the stem-leaf,  $\times 210$ . G. Branch-leaves,  $\times 17$ . H. Outer surface of branch-leaf showing fibres and pores,  $\times 210$ . I. Inner surface of branch-leaf,  $\times 210$ . Chl. Chloroplastids. J. Portion of section of branch-leaf showing two hyaline and one chlorophyllous cell,  $\times 210$ .

The formation of capillary chambers along which water may pass is general in the genus. The branches are in fascicles (Fig. 2, A), their number varies in different species, and what is more important, they vary in position, some being stronger than others so that they occupy more patent positions. The weaker branches are often closely appressed to the stem and so form

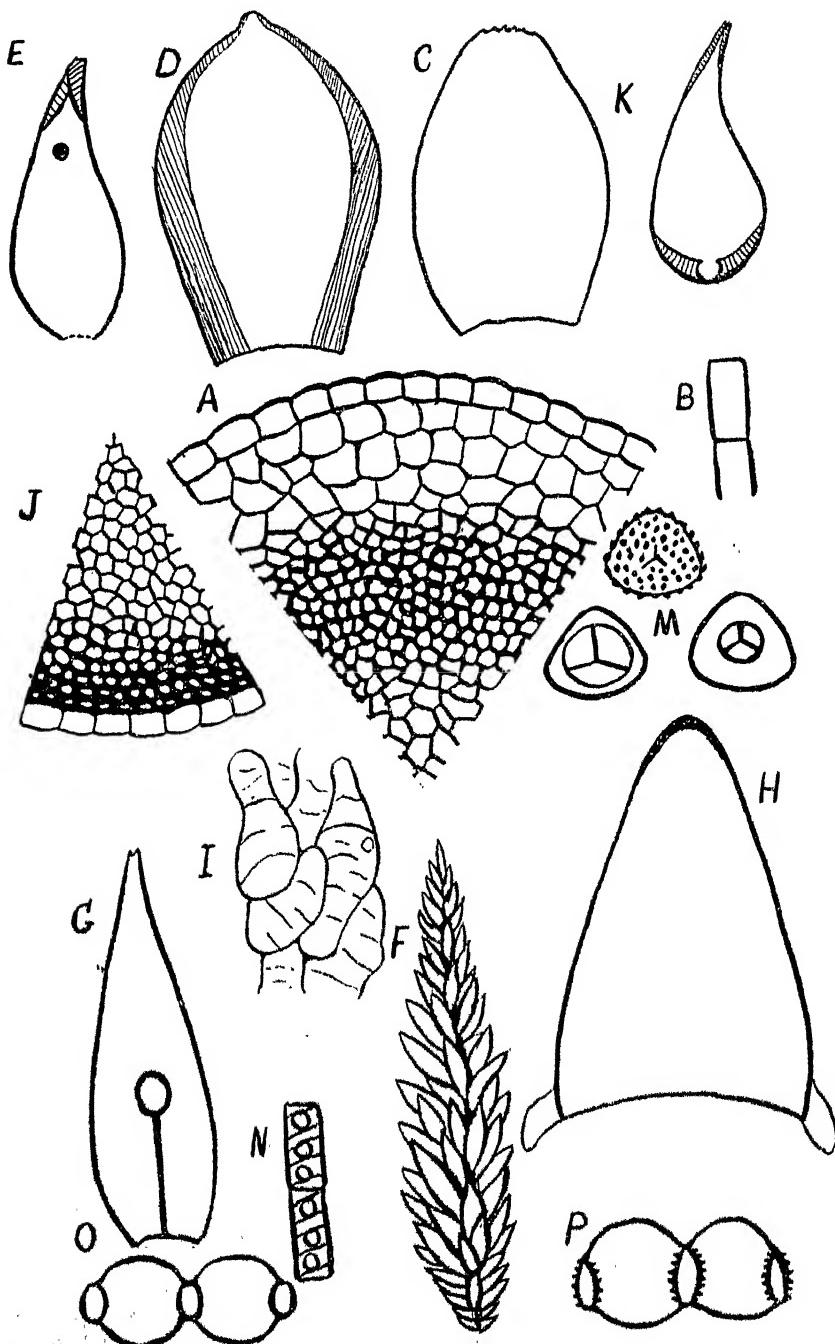


FIG. 3. (See description on opposite page.)

fine channels along which water passes by capillary action. In most species there are two or three divergent and one or two pendent branches (Fig. 2, B). In the two allied plants *S. cuspidatum* and *S. recurvum*, the former is a plant with a more characteristic aquatic environment, and this is reflected in the almost complete absence of pendent branches, especially in the wholly-immersed variety *plumosum* (Fig. 4, A), whilst the stem of *S. recurvum* is concealed by them, two or three of the four or five fascicled branches being pendent (Fig. 5, A). *S. squarrosum*, which, as its name implies, has squarrose leaves, often grows in shaded places, but when growing in open places has, like *S. recurvum*, two or three pendent branches which enable water to be drawn up from the constantly moist substratum. In the usually immersed *S. riparium* the pendent branches are scarce or absent, and in *S. obesum*, the most aquatic member of the Subsecunda group, they are wanting.

The intermixture in the branch-leaf of empty hyaline cells with those containing protoplasm and chlorophyll is a generic character. The hyaline cells are provided with small openings or pores, strengthening fibres prevent collapse, and generally they are much wider than the alternating chlorophyllous cells. They are regarded as water-reservoirs with a special aptitude for obtaining a quick and copious supply of water, and there is no reason to doubt this explanation of their presence, but the relative orientation of the two kinds of cells, though constant for a particular species, is variable in the genus, and in some cases is suggestive of a protective device against undue loss of water from the living cells. It is also significant that in some submerged species, varieties, and forms, the hyaline cells are reduced or are replaced by chlorophyllous ones. In the submerged species, *S. obesum* and *S. riparium*, many of the cells, which are structurally hyaline, contain chlorophyll, and in a submerged form of *S. imbricatum* (form *degenerans*) the chlorophyllous cells are so much broadened that the hyaline cells sometimes seem to be absent.

FIG. 3. *Sphagnum subnitens*. A. Portion of transverse section of stem,  $\times 100$ . B. Surface view of two of the external cells of the stem,  $\times 100$ . C. Perichaetial leaf,  $\times 16$ . D. Perichaetial leaf indicating the border of narrow cells,  $\times 16$ . In the middle of the leaf the hyaline and chlorophyllous cells are distinct; in the lower part they are indistinctly differentiated; in the upper part the cells are smaller, relatively broader, and not differentiated. The hyaline cells have no fibres or pores and are one—or more—septate. E. Branch, bearing antheridia in the middle portion,  $\times 10$ . F. Leaf and antheridium,  $\times 32$ . G. Leaf and antheridium,  $\times 400$ . The upper shows the papillose surface, the two lower show the triradiate marks.

*S. rubellum*. E. Branch-leaf,  $\times 30$ .

*S. inundatum*. H. Stem-leaf with auricles,  $\times 30$ . I. A few cells from the auricles showing incomplete or weak fibres,  $\times 200$ .

*S. rufescens*. J. Transverse section of stem,  $\times 100$ .

*S. subsecundum*. K. Branch-leaf,  $\times 30$ . O. Section of branch-leaf,  $\times 400$ , showing two hyaline and two chlorophyllous cells.

*S. cymbifolium*. N. Surface view of two of the outer cells of the stem showing fibres and pores,  $\times 400$ .

*S. papillosum*. P. Transverse section of branch-leaf,  $\times 400$ . The outer surface is uppermost in the figure.

All the leaves are figured as viewed from the inner surface.

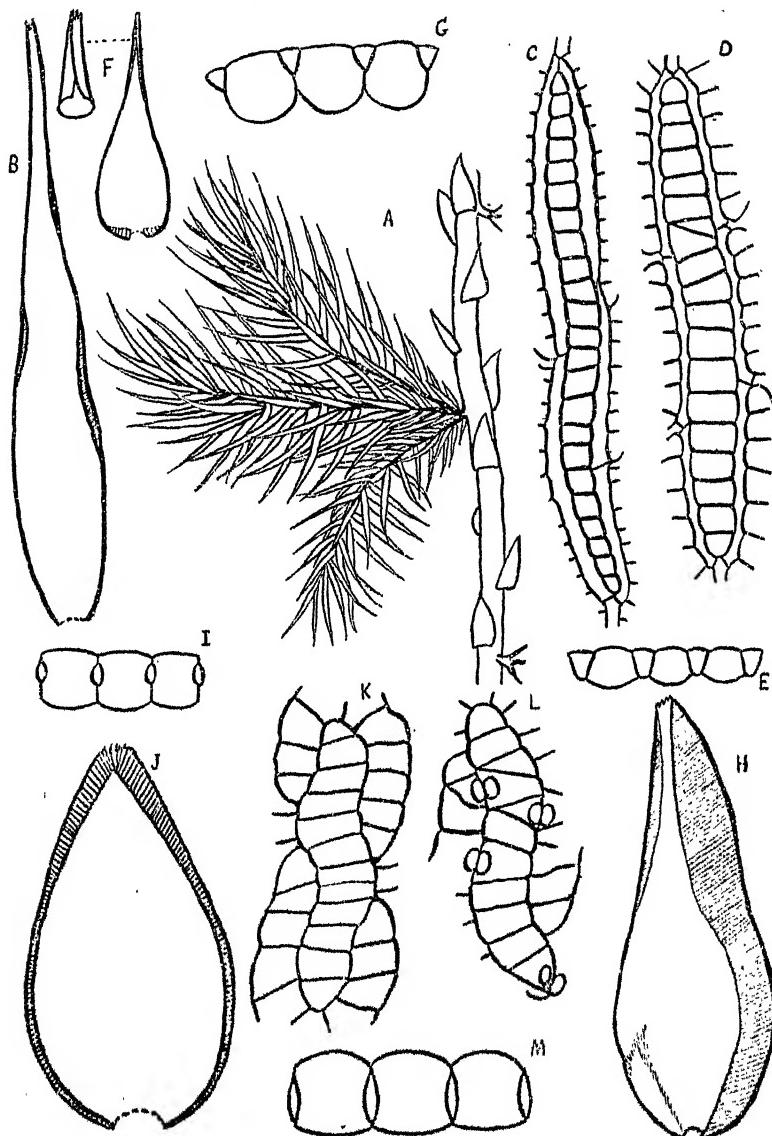


FIG. 4. *Sphagnum cuspidatum* var. *plumosum*. A. Portion of the plant showing a fascicle of branches,  $\times 5$ . The positions of two other fascicles are indicated. B. Branch-leaf,  $\times 23$ . C. Cells of the branch-leaf viewed from the outer surface,  $\times 300$ . D. Cells viewed from the inner surface,  $\times 300$ . E. Transverse section of branch-leaf,  $\times 300$ .

*S. pulchrum*. F. Branch-leaf,  $\times 23$ ; apical portion forming a tube,  $\times 45$ . G. Transverse section of branch-leaf,  $\times 300$ .

*S. compactum*. H. Branch-leaf,  $\times 23$ . I. Transverse section of branch-leaf,  $\times 300$ .

*S. medium*. J. Branch-leaf,  $\times 23$ . K. Cells as seen from inner surface of branch-leaf,  $\times 300$ . L. Cells from outer surface,  $\times 300$ . M. Transverse section of branch-leaf,  $\times 300$ .

All the leaves (unless otherwise stated) are figured as seen from the inner surface. The incurved portions of the leaves are shaded. In a figure of a leaf-section the outer surface is placed uppermost.

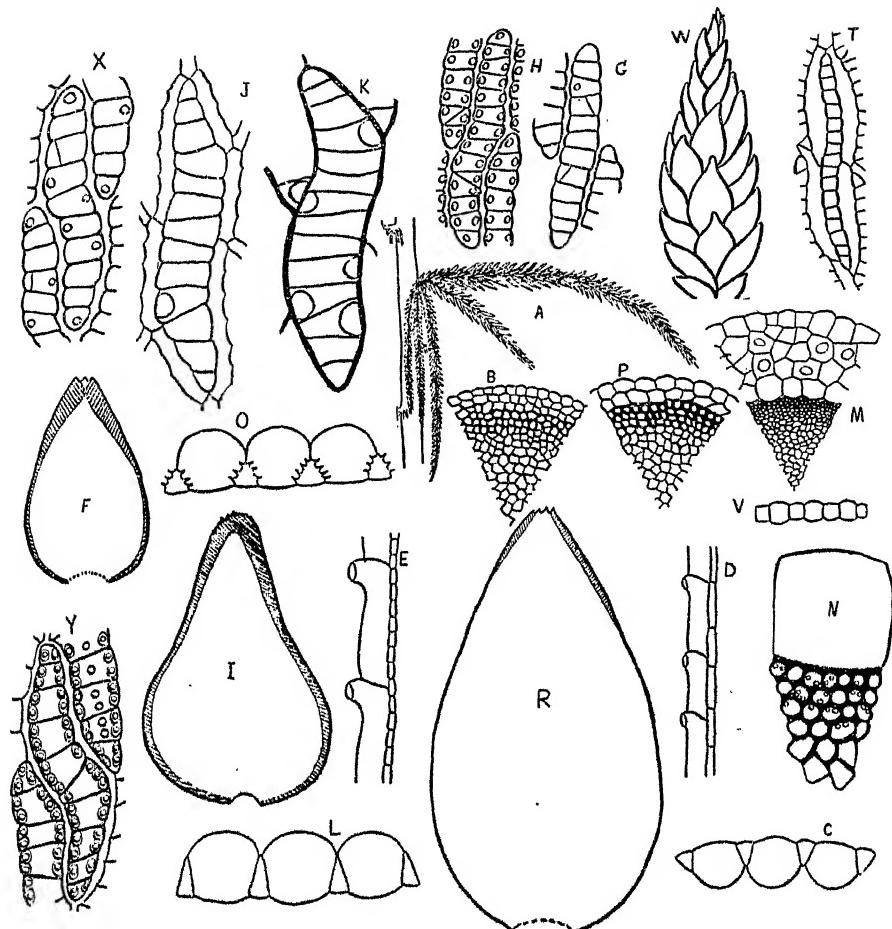


FIG. 5. *Sphagnum recurvum*. A. Portion of plant showing a fascicle of branches, three other fascicles being indicated. The figure is somewhat diagrammatic, since the pendent branches have been separated from each other and from the stem, whilst those from the other fascicles have been removed,  $\times 4$ . B. Transverse section of stem,  $\times 60$ . C. Transverse section of branch-leaf,  $\times 240$ .

*S. amblyphyllum* ( $\sim$  *S. recurvum* var. *amblyphyllum*). D. Outer portion of branch, showing the layer of retort cells and one layer of cortical cells,  $\times 80$ . All the leaves have been removed.

*S. molluscum*. E. Outer portion of branch, showing large retort cells and smaller cortical cells,  $\times 80$ .

*S. inundatum*. F. Branch-leaf,  $\times 18$ . G. Cells of branch-leaf as seen from the inner surface,  $\times 240$ . H. Cells of branch-leaf from the outer surface,  $\times 240$ .

*S. cymbifolium*. I. Branch-leaf,  $\times 18$ . J. Cells from inner surface,  $\times 240$ . K. Cells from outer surface,  $\times 240$ . The chlorophyllous cells are almost concealed by the hyaline cells, the walls of which appear to be thick, this appearance being due to the nearness of the two walls, whilst the darker contents of the chlorophyllous cells just show between. L. Transverse section of branch-leaf,  $\times 240$ . M. Transverse section of stem,  $\times 60$ . N. Portion of stem-section showing one hyaline cell and part of the stereom cylinder,  $\times 240$ . Chloroplasts are present in some of the cells.

*S. imbricatum*. O. Transverse section of branch-leaf,  $\times 240$ .

*S. cuspidatum*. P. Transverse section of stem,  $\times 60$ .

*S. obesum*. Q. Branch-leaf,  $\times 18$ . T. Cells of branch-leaf viewed from inner surface,  $\times 240$ . V. Transverse section of branch-leaf,  $\times 240$ , showing four chlorophyllous and three hyaline cells.

*S. compactum*. W. Branch showing imbrication of leaves,  $\times 7$ .

*S. rufoescens*. X. Cells of branch-leaf viewed from inner surface,  $\times 240$ . V. Cells from outer surface,  $\times 240$ .

All the leaves are figured as seen from the inner surface. The incurved portions of the leaves are shaded. In a figure of a leaf-section the outer surface is placed uppermost.

The orientation of the two kinds of cells, their relative sizes, shapes, and porosity, will be discussed in a more detailed manner later.

Papillosity of the cell-wall (Fig. 3, P) is a character met with in few species (*S. papillosum*, *S. imbricatum*). In *S. medium*, *S. squarrosum*, *S. teres*, and *S. Wulfianum* papillae are sometimes met with, but they are scarce or very minute. In some varieties of *S. papillosum*, which are generally plants of wetter situations than the type, the papillae are absent (var. *leve*) or very minute and only met with in some of the leaves (var. *subleve*). *S. imbricatum* has longer papillae (Fig. 5, O), but has not a drier habitat; in some other respects (e.g. greater width of chlorophyllous cell in respect to the hyaline one) its structure is more hydrophytic. Its variety *affine* has its cell-walls devoid of excrescences and is found in more constantly wet places.

Many examples of chambers for the storage of water have been mentioned; in addition the outer layers of the cells in stem, the spaces between the stem-leaves and the stem, the swollen auricular cells at the basal angles of the stem-leaves, the retort cells of the branches, and the spaces between the imbricated and concave leaves often serve for storing water. Some of these will be referred to with more detail later on.

Many *Sphagna* have a compact or tufted habit which is more pronounced in plants of drier moorlands, e.g. *S. compactum*, *S. cymbifolium* var. *congestum*.

#### REASONS FOR PROTECTIVE DEVICES.

It is difficult to reconcile the general watery habitat of *Sphagnum* with the presence of characters which, in other Mosses, act as xerophytic devices, and, as is evident from the comparison of different kinds of *Sphagnum*, also tend to act as protective devices with them. This tendency, however, is not always manifest; inconsistencies and anomalies are apparent if we attempt to make a comparative correlation of the structure of the plants and the wetness of their habitats. A species which is often found in a moist place, where it may be exposed to temporary dryness, is also found in a bog pool where wetness is permanent or almost so; a species showing many of the foregoing devices may occur in an almost constant pool, whilst another with fewer or less prominent devices is in a temporary water-basin, so that one is almost tempted to conclude that there is no correlation, and that the species of *Sphagnum* have wide ranges of habitat. Careful field-work, however, shows that there is some selective power, some species favouring very wet, and others moist habitats; but still there must be other factors influencing the distribution besides the degree of wetness, and the most important of these is the acidity of the solution.

It seems highly probable from recent work that *Sphagna* produce acids owing to the adsorption of the base by some substance of a colloidal nature present in the cell-walls, the amount of acid liberated being

a character which is fairly constant for a particular species under similar conditions. Owing to this method the plant is able to obtain its mineral food from very dilute solutions, and the author has long held the view that it can only grow well after fresh water has been supplied.<sup>1</sup> It may be submerged in water that is useless for the due discharge of its physiological functions since it is too acid in character and not available owing to its high osmotic activity; it is only when the solution is considerably weakened (as after rain) that the plant resumes its normal growth, the death and decay of the cells in the lower portions of the plant being largely due to the inability of the cells to use the water after it has become strong in 'humic' substances by concentration during a period of dryness.

*Sphagnum* seldom occurs in standing pools and stagnation of the water eventually kills it. It may occur in deep bogs, but the cells which are normally active are those towards the surface, and therefore in a position to profit more directly by the influx of dilute solutions. The normal habitat of many species, c. g. *S. cymbifolium*, *S. papillosum*, *S. acutifolium*, is on the margins of bogs, or in other places where an influx of fresh water is readily perceived by them. *Sphagnum* is a plant which has rapid growth in suitable conditions, and if very dilute solutions supply the mineral and nitrogenous substances necessary for metabolic processes, a large amount of these solutions will be required, and it is possible that

- (1) the solution is absorbed by the plant and the redundant water got rid of (*a*) by excessive transpiration from the chlorophyllous cells, or (*b*) by evaporation from the hyaline cells;
- (2) there is a special method whereby a colloidal substance present in the cell-wall adsorbs the base of the mineral salt.

When the plant relies mainly on the latter method the surrounding water becomes acid and may so interfere with endosmosis that sufficient water for physiological purposes is not available, and so devices protective against loss of water may occur. The structure of the plant is such that available water is quickly taken in by the hyaline cells, and passage of water up or down the surface of the plant is facilitated. For example, in *S. cymbifolium*, the hyaline cells with pores on their outer surfaces will quickly absorb the water during or after rain, whilst the pendent branches preserve a watery communication for diffusion of the mineral salts to the cells and of the liberated acids from the cells.

This special method does not exclude the more normal methods of obtaining mineral substances, is possessed by all *Sphagna* to some extent, and in most species appears to be the chief method. We can roughly place the species in three groups:

<sup>1</sup> Goebel, in *Organography of Plants*, has a note on the habitat of *Sphagnum*. He says, 'Sphagna chiefly live upon rain-water; they grow in places where the water only contains a small amount of mineral substances necessary for their nourishment, so that a profuse water-evaporation is necessary'.

1. Species which obtain their mineral substances from the water in which they live, have no excessive transpiration or evaporation, but supplement their income by the special method. They are usually plants of slow growth completely immersed in water. Their acidic value is low and their structure is the nearest to the phylogenetically primitive form. *S. obesum* is the best example of this group, but some other members of the Subsecunda section approximate to it.
2. Species which obtain their mineral substances by excessive transpiration of the redundant water from the exposed chlorophyllous cells, and also add to their income by the special method. They are plants of constantly moist situations, have a good supply of pendent branches for the passage of water to the exposed portions (Fig. 5, A), their acidic value is usually low and their chlorophyllous cells have been displaced to the outer sides of the leaves. *S. recurvum* and *S. amblyphyllum* belong to this group, whilst some other allied species closely approximate to it. *S. squarrosum* is better placed with this group, though in some respects it approaches the next.
3. Species which usually obtain most of their mineral substances by adsorption of the base and liberation of the acid. They are plants of variable habitat, their acidic values are usually high, and their structure is greatly modified. The most definite members of this group belong to the Acutifolia (e.g. *S. rubellum*) and Cymbifolia (e.g. *S. medium*) sections. They may sometimes be partially immersed in acidic water, whilst at other times they are much exposed, so that devices protective against dryness are adopted, these devices being chiefly for the purposes of facilitating intake and passage of water rather than against loss of water.

Some species have varieties which may be more appropriately placed in another group; thus the variety *imbricatum* of *S. squarrosum* is nearer to the third than to the second group, in which the type may be placed.

The species of the third group obtain their mineral salts from very dilute solutions formed after rain, those of the first group from the dilute solutions in which they live (the solids in siliceous streams are often less than 0·1 grm. per litre), whilst the second would usually be able to obtain a rather stronger solution. The following results were obtained on evaporating the water squeezed out from the tufts of *Sphagnum*:

<i>Species.</i>	<i>Group.</i>	<i>Habitat of Example tested.</i>	<i>Solid in Grm. per Litre.</i>
<i>S. cymbifolium</i>	3	Moist hollow of heath	0·05
<i>S. cymbifolium</i>	3	Damp hollow of heath	0·09
<i>S. Girgensohnii</i>	3	Moist places of heath	0·07
<i>S. rufescens</i>	1	Stream side just above the water, a constantly wet place	0·11
<i>S. subnitens</i> , mixed with a little <i>S. cymbifolium</i>	3	Stream side, but some distance above the water	0·08
<i>S. amblyphyllum</i>	2	Wet place on heath, a constantly moist place	0·13

FURTHER CHARACTERS IMPORTANT IN RELATION  
TO WATER-SUPPLY.

Besides the characters previously mentioned there are others which are worth considering in relation to the water-supply :

(1). *The relative positions, shapes, and sizes of the chlorophyllous and hyaline cells*, particularly of the branch leaves. (a) In *S. subsecundum* and its allies the two kinds of cells are almost rectangular in shape; the chlorophyllous cell is medianly placed between the contiguous hyaline cells, and is more or less exposed on both the inner and outer surfaces of the leaf (Fig. 3, K ; Fig. 5, V), so that the only protection afforded to it by the hyaline cells is due to the fact that the thickness (antero-posterior diameter) of the hyaline cell is greater than that of the barrel-shaped chlorophyllous one and so to some extent overlaps it, the surface of the latter being less than the average area of a cross-section. The species of this section are founded on differences between the characters of the upper branch leaves; the lower branches are usually immersed in water, have less distinctive characters, their chlorophyllous cells are wide, more or less rectangular, and little overlapped by the hyaline cells. In *S. obesum*, the most hydrophytic member, there is often little difference in size, shape, and position of the two kinds of cells (Fig. 5, V).

The median position of the chlorophyllous cell is also found in *S. papillosum* (Fig. 3, P) and *S. medium* (Fig. 4, K, L, M), plants of higher acidity and drier situations, but the chlorophyllous cell is more enclosed by the hyaline, and other methods (e. g. imbricated and cucullate leaves) for retaining or obtaining water are more prominent in these plants.

(b) In *S. cuspidatum* (Fig. 4, E), *S. squarrosum*, and their allies the chlorophyllous cell is triangular to sub-rectangular with its broader end exposed on the outer surface of the leaf. The outer walls of the hyaline cells are slightly convex but afford little shade to the surface of the chlorophyllous cell; the inner walls are often more convex and overlap the chlorophyllous cells, but little protection against normal desiccation is afforded by this structure, so that practically the only advantage accruing to the living cell by the proximity of the hyaline one is that the latter acts as a reservoir. Under strong desiccating influences evaporation may occur on both the inner and outer surfaces and then the enclosure of the chlorophyllous cell on the inner surface may be beneficial.

*S. cuspidatum* (Fig. 4, E), with large sub-rectangular chlorophyllous cells free on both surfaces of the leaf, has the most aquatic environment; *S. pulchrum* (Fig. 4, G), with the cells partially enclosed, has the driest habitat; whilst *S. recurvum* (Fig. 5, C) and *S. squarrosum* are intermediate both in structure and habitat. *S. compactum* has its chlorophyllous cells

almost median but nearer to the outer surface (Fig. 4, I). Its densely tufted and compact habit, its closely imbricated and slightly cucullate leaves, enable it to grow in drier situations than any of the above, though its var. *squarrosum* with less crowded branches and more or less squarrose leaves has a wetter habitat than the type.

(c) In *S. cymbifolium* (Fig. 5, J, K, L), *S. imbricatum*, *S. fimbriatum*, and the Acutifolia group (Fig. 2, J), the chlorophyllous cells are triangular in shape, the base of the triangle being on the inner side of the leaf, whilst the outer walls of the hyaline cells are so convex that they entirely or almost entirely enclose those containing chlorophyll. This arrangement is an excellent device for lowering the illumination and for preventing excessive transpiration, and the Sphagna which possess it grow in situations exposed to light and with an inconstant water-supply, or in water which is strongly acid at times. These plants are seldom shaded; if they are, compensating characters such as looser or squarrose leaves (e.g. *S. cymbifolium* var. *squarrosum*) are shown. As the Sphagna with median cells are more primitive in this respect, the displacement to the outer or inner surfaces may be regarded as due to light as well as to water requirements.

The relative sizes of the two kinds of cells must also be considered. The water-supply will be more profuse when the ratio

$$\frac{\text{Volume of hyaline cell}}{\text{Volume of chlorophyllous cell}}$$

becomes greater if we consider cells with similar positions and shapes, and when there is any overlapping of the hyaline cell over the chlorophyllous one, the shading will be greater the narrower the latter is; in *S. obesum* and *S. medium* the chlorophyllous cell is median, but is wider in the former, a plant of wetter habitat (cf. Fig. 5, T, V; and Fig. 4, K, L, M).

The stem-leaves also have variations similar to those of the branch-leaves, though of much less extent (Fig. 2, C, D, E, F). As they are usually concealed by the branch-leaves they have less chlorophyll; there is often little differentiation between the two kinds of cells, pores and fibres are fewer or absent, and in most cases their chief function is to assist the pendent branches in supplying water to the upper parts of the plant. The perichaetal and perigonial leaves are often more primitive in their structure and the differentiation into two kinds of cells is often incomplete (Fig. 3, C, D, F, G).

#### 2. Position, size, number, and bordering of pores in the hyaline cells, especially in the leaves of the upper divergent branches.

The hyaline cell usually possesses fibres to maintain its shape, and its membrane is usually perforated to form pores. The usual form of pore is surrounded by a distinct thickened ring of cell-membrane which keeps it open, but non-bordered perforations also occur, especially on the lower portions or the inner surfaces of the leaf. The chief function of these pores

is the rapid intake of water, but they are also of use in promoting a readier diffusion of acids, and in some cases (especially in the Subsecunda section) a considerable amount of evaporation may occur through the pores on the outer surface. Their disposition varies according to the species:

(a) Pores chiefly on the outer surface of the branch leaf.

1. Pores large and few in each cell:—*Cymbifolia* section (Fig. 5, J, K; Fig. 4, K, L). In *S. squarrosum* and *S. teres* the pores are large and few, but the porosity of the inner is similar to that of the outer surface.

2. Pores moderate and few in each cell:—*Acutifolia* section (Fig. 2, H, I), *S. compactum*.

3. Pores small and many in each cell:—*S. subsecundum*, *S. inundatum* (Fig. 5, G, H), *S. rufescens* (Fig. 5, X, Y), *S. auriculatum*. In *S. rufescens* the pores are often numerous on the inner surface as well.

(b) Pores small and many, chiefly on the inner surface of the branch leaf:—*S. crassicladum*, *S. molluscum*. In the former the outer surface shows thickenings (pseudopores), indicating that it has been evolved from an ancestor possessing pores on the outer surface.

(c) Pores absent or few on both surfaces, if present small and usually on the inner surface:—*S. cuspidatum*, *S. riparium*, *S. obesum*, *S. recurvum*, *S. amblyphyllum*, all of which have an abundant water-supply (Fig. 4, C, D; Fig. 5, T).

The pores on the outer surface facilitate the rapid intake of surface water (as after rain), are usually bordered and so kept open. Evaporation of water may occur through them, and will be greater when the pores are numerous and small than when few pores have an equal area. When the leaves are more or less imbricated the pores on the upper exposed portions of the outer surface will be more efficient for obtaining external water, and these places show the most characteristic pore formation. The pores on the inner surface are favourably situated for interchange of substances and for obtaining water from the dilute solution passing along the axis of the plant; they are present though few and scattered in *S. recurvum* and *S. squarrosum* (with large pores), which obtain their main water-supply from below, but are best shown in the upper branches of *S. crassicladum*, a plant which is often immersed or almost immersed in streamlets or pools of fresh water.

The pores on the inner surface of the leaf or on the lower part of the outer surface overlapped by an imbricated lower leaf are usually weakly- or non-bordered, are often larger, and allow ready interchange of substances with the axial water-supply. The absence or paucity of pore formation is correlative with an abundant water-supply.

3. *Large superficial hyaline cells of the stem.* The presence of these may be considered a generic character, as in all species there are one or more cortical layers of large, thin-walled, hyaline cells surrounding a stereom ring of smaller and thicker walled cells and a central core of thin-

walled and fairly large cells. The only cells containing chlorophyll are immediately underneath the superficial hyaline cells. The following types occur:

- (a) The hyaline cells are in two or three layers but are not very distinct from the more internal cells, being only slightly larger and thinner walled:—*S. recurvum* (Fig. 5, B), *S. amblyphyllum*, *S. obtusum*. In *S. riparium*, *S. compactum*, and *S. pulchrum* they are often somewhat distinct, and therefore these plants may be better placed in (c).
- (b) They form one layer of large cells (with few or no pores) clearly differentiated from the stereom cylinder:—*S. subsecundum* and its allies (Fig. 3, J).
- (c) They form two to five layers of large cells clearly differentiated from the stereom cylinder:—*S. acutifolium* and its allies (Fig. 3, A), *S. squarrosum*, *S. teres*, *S. cuspidatum* (Fig. 5, P), *S. molluscum*, *S. cymbifolium* (Fig. 5, M) and its allies. Pores are few or absent (Fig. 3, B) except in the Cymbifolia group, which also have strengthening fibres in the cells (Fig. 3, N).

The plants of the first two types have an abundant water-supply, and have a low acidity, whilst most of the plants of the third type have a high acidity, and at times are exposed to drier conditions. The hyaline cells of the stem and their contained pores have similar functions to those of the leaf. The fibres similarly serve as strengthening bodies which cause the cell to retain its shape so that water may be quickly absorbed. In the leaf the fibres are often weak or absent in parts where there is a constant water-supply. Other things being equal the plants with pores and fibres are more able to withstand exposure.

4. *Other hyaline cells for the storage of water* are present in the retort cells and leaf auricles.

The retort cells are large flask-shaped cells superficially placed on the outside of the hyaline cells of the branch; their necks often curve away from the axis and are perforate at the ends. They vary according to the species, sometimes being absent, and reach their highest development in *S. molluscum* (Fig. 5, E, cf. D).

The basal angles of the stem-leaf are usually occupied by larger hyaline cells, and sometimes these are extended into little outgrowths known as auricles (Fig. 3, H, I). e.g. *S. auriculatum*, *S. inundatum*.

#### SPOROGONIAL PLANT.

As the sporogonium is overtopped by the perichaetial leaves till it is almost mature, little further protection is needed by it. The calyptra is small and quickly disappears when the false seta elongates. The sporogonia then soon begin to 'pop' and the spores are scattered. In some species the

spores are shed when the plant is exposed to drier conditions than usual, and this may account for the papillosity of the spores (Fig. 3, M).

#### ASSOCIATES.

The associates of *Sphagnum* will be dealt with in a later paper.

#### GENERAL CONCLUSIONS.

*Sphagna* obtain their mineral salts from very dilute solutions.

They possess a special method of obtaining mineral food by adsorbing the base and liberating the acid.

In order to do this, special devices (which may seem of a xerophytic nature) are adopted to obtain a sufficient quantity of dilute solution, and to get rid of the superfluous acid and water.

These devices vary in different groups of *Sphagnum*. During periods of drought the income of exposed plants is suspended and the xerophytic devices may act as such, keeping the plant in a moist condition till a further supply of dilute solution is available.



# A Study in the Anatomy of Hazel-wood with Reference to Conductivity of Water.

BY

M. G. HOLMES, B.Sc.

With two Figures and eight Graphs in the Text.

THE work described in this paper was carried out under the direction of Professor Farmer at the Imperial College, the aim being to find an anatomical basis for variations in the conducting power of wood for water. Professor Farmer has shown experimentally, in his recently published papers,<sup>1</sup> that the wood in different regions of the same shoot may vary considerably in specific conductivity; that is, in the conducting efficiency calculated for equal areas in transverse section and equal lengths. The total conducting power of the wood at any one position in the shoot depends partly upon the area of the wood in transverse section, but is influenced also by the specific efficiency, or quality, of the wood. In one shoot the total amount of water to be transmitted through the wood decreases from near the base upwards; if the wood were equally efficient as a water-conducting medium in all parts, the decrease in the area of the transverse section would be a measure of the decrease in conductivity. But the wood is by no means equally efficient as regards this function; and therefore comparison of the amount of wood in transverse section is not sufficient to explain differences in the total amount of water passed through in a given time: a further explanation must be sought in differences in constitution. Thus variation in specific conductivity must be related to variation in the number, size, character, and distribution of those elements in the wood through which the water passes.

Considered as a whole the wood has more than one function. Through the tracheae or vessels, and the tracheides, it conducts water; but these water-conducting elements are associated with woody fibres having a mechanical function only, and also with living cells. During autumn and winter the latter are filled with starch, and they may be classed together as storage tissue. I shall not enter here into the question of how far the living cells help in the water-conducting process: for the purpose of comparing water-conducting efficiency I have taken into consideration only the character

<sup>1</sup> J. B. Farmer: On the Quantitative Differences in the Water-Conductivity of the Wood in Trees and Shrubs, Parts I and II. Proc. Roy. B. Soc., vol. xc., 1918.

and distribution of the vessels and tracheides. Since part of the wood consists of elements serving a mechanical purpose only, it is clear that an increase in the proportion of this part, in any regions of the shoot requiring extra support, will result in a decrease in the proportion of the other parts; that is, without disturbing the necessary amount of total conductivity, such a condition will decrease the specific conductivity. From this point of view

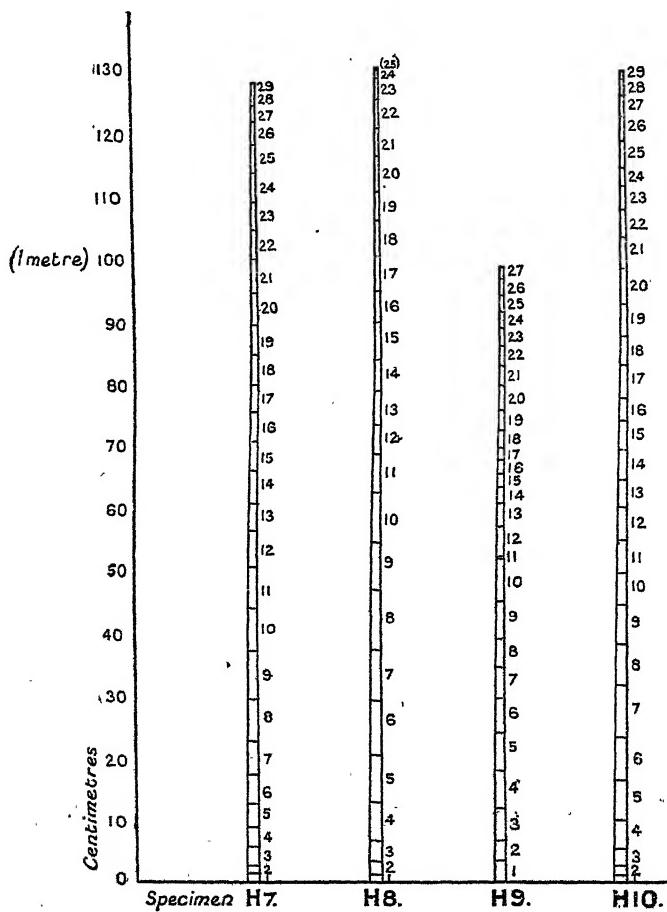


FIG. 1.

I have examined a number of similar shoots, to discover the variation in the proportion of water-conducting elements in the wood, by observations on their size and distribution; and in this way to provide data for an anatomical explanation of the variation in specific conductivity.

*Material.* For this investigation the material selected consisted of stool shoots of the hazel in their first year of growth. It is well known that the shoots which spring up from a stool in the first year after coppicing are

extremely vigorous; they attain a much greater length and thickness than the shoots borne on old branches of the tree, and they bear abnormally large leaves. While they exhibit great vigour in the direction of vegetative growth, they do not bear reproductive organs, no inflorescences being formed in the winter buds. In fact the stool shoots differ sufficiently from the tree shoots to be distinguished as a juvenile form. The rapid growth of these shoots and the large size of their leaves might suggest that they are provided with an unusually efficient water-conducting system, but it is clear from Professor Farmer's experiments that the wood in stool shoots has on the average a lower specific conductivity than that shown by shoots taken from older parts of trees of the same species, while there is also a considerable and regular variation in specific conductivity in different regions of the same shoot. Anatomical investigation should indicate the extent and perhaps throw light on the cause of this variation.

The four shoots investigated were cut on September 22, 1917, from separate stools in the same wood, coppiced the previous winter; they were measured, catalogued, and preserved in 70 per cent. alcohol. At this time in autumn the full number of internodes has been formed, and the apex for the winter is determined; development in length and width is almost complete, but the free end of the shoot has not reached its ripened winter

condition, and the leaves are still functional. In the hazel the more vigorous stool shoots reach a length of over  $1\frac{1}{2}$  metres in their first season, and some of them branch from a few of the upper nodes. The particular shoots chosen were unbranched, and were all a metre or more in length, being composed of from twenty-four to twenty-nine internodes. The lengths of these and of the whole shoots respectively are compared in Fig. 1, from which it will be seen that the longest internodes occur in the lower middle part of each shoot. The leaves are largest in this region also; the largest leaf measured was 13 cm. long and 11.5 cm. wide. In September four or five of the nodes at the base of the shoot have lost their leaves. In most cases, but not in all, the true terminal bud aborts, and the apex of the shoot is occupied by a lateral bud. In one of the specimens examined, H8, the

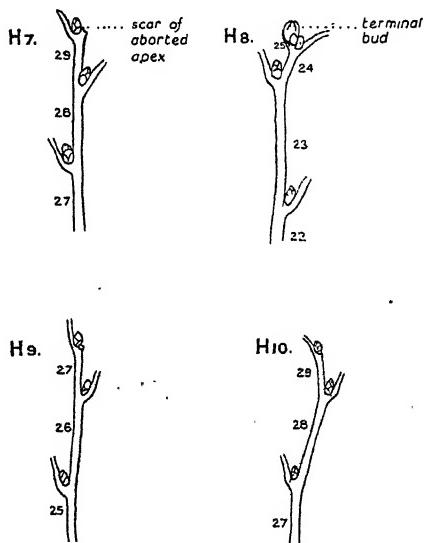


FIG. 2.

terminal bud was present; this is shown in Fig. 2. By reference to Fig. 1 it will be seen that, of these four specimens, H8 showed the smallest number of internodes for the greatest length.

*Anatomy.* The most apparent difference between the stool shoots and tree shoots as regards the internal arrangement of tissues consists in the much larger size of the pith in the former. It maintains a large size all through the middle part of the shoot, decreasing slightly towards the apex, and more rapidly towards the base, where it becomes quite small. The decrease in the width of the layer outside the cambium, consisting of phloem, cortex, and cork, is but slight, so that in the large transverse sections of the shoot, near the base, there is a disproportionately large amount of wood, compared with the smaller sections higher up the same shoot. The tissues in stool shoots and tree shoots in their first year are present in fairly similar proportions, but on the whole there is a greater proportion of pith and of wood in the former. The cylinder of wood decreases in width from base to apex; it is hard and firm in the lower part of the shoot and becomes softer in the upper part. In the terminal internode the cylinder is not completely closed, being still interrupted by wide medullary rays. In sections from the terminal internodes of similar stool shoots cut in February the wood is more mature and the cylinder has become closed.

The wood consists of dead lignified elements interspersed with living cells and divided radially into narrow sectors by medullary rays also composed of living cells. Near the apex of the stem the dead elements are almost all of a kind capable of conducting water, but lower down a large number of them are fibrous, while towards the base the fibres predominate greatly, the water-conducting elements being scattered sparsely among them. The elements formed first, i. e. next to the pith, are vessels with spiral thickening, and constitute the protoxylem; these can be distinguished at all levels in the stem. In the upper part of the stem they carry a considerable proportion of the water, but lower down they become crushed, and their functions are assumed by the pitted vessels which are formed later. The vessels of the primary xylem have spiral thickening also; they are arranged in radial rows, and increase in diameter from the protoxylem outwards. At all levels in the shoot this region of the wood, immediately outside the pith, is richest in vessels. They are crowded together, with only a few fibres between them here and there. This represents the response of the wood to the first demand of the growing stem for water, and it is not till later that the wood begins to exercise its supporting function, and mechanical elements become differentiated in larger proportion. The widest vessels occur in the inner part of the secondary xylem. In the lower part of the stem the narrower vessels towards the periphery are associated with tracheides. Here the water-conducting elements are distributed in obliquely radial groups, the spaces between being occupied by mechanical and storage tissue

only. The tracheides are, on the average, narrower than the vessels, but their walls are similar, so that in transverse section they cannot be distinguished from small vessels with absolute certainty.

From the fibres both vessels and tracheides are distinguishable in transverse section by the character of their walls. Those of the fibres are relatively thick and show few obvious pits, while those of the water-conducting elements are thinner and provided with bordered pits; the latter also stain more deeply with safranin. In longitudinal section the vessels are seen to have walls studded with numerous small bordered pits, and their members communicate by perforations in the transverse walls. These walls lie obliquely in the radial plane, the perforations taking the form of a single row of large open pits in each. The tracheides are without open pits; they have pointed ends, and their length is thirty to forty times their diameter; on the average they are shorter than the vessel members. The fibres have narrow tapering ends and a few small oblique pits; their length on the average also exceeds that of the tracheides, especially in the lower part of the stem, where the largest reach a length of 1 mm. and a width of  $20 \mu$ .

The storage tissue in the wood consists of cells with lignified and pitted walls and protoplasmic contents; they occur in scattered vertical rows, and in bands crossing tangentially from one medullary ray to the next; the individual cells are elongated more or less in the vertical direction. The cells composing the medullary rays are similar in structure, but somewhat elongated radially. The rays consist of single, or sometimes double, radial rows of cells, and vary in height from one to fifty or more cells, as seen in tangential section. All the water-conducting elements are in contact with living cells.

From the point of view of water-conducting efficiency special attention must be given to the proportion of the area of the wood in transverse section which is occupied by vessels and tracheides; but conductivity may be influenced also by the proportion of vessels to tracheides, and by variation in the average length of the whole vessels. Since specific conductivity is measured in terms of the amount of water passed through at a given pressure in a given time, it must be affected by resistance. Vessels will offer less resistance than tracheides, in which the water has to pass more often through the pit membranes in the end walls; and for the same reason long vessels will offer less resistance than shorter ones of the same diameter. Thus the elements in question must be compared both in their transverse and in their longitudinal characters.

*Method.* In order to put into a definite form these general ideas of the structure of the wood, and its variation in different parts of the stem, I have collected a number of data relating to the size and distribution of the water-conducting elements. Each shoot was examined in the following way. The internodes having been numbered from the base upwards (Fig. 1), transverse

sections were made at the centre of each of a number of particular internodes chosen at various heights along the shoot. Drawings of these sections were made on millimetre squared paper, by means of the camera lucida, and the total areas of the section as a whole and of the wood in the section were calculated. Then, in a typical section of the wood, the area of which was determined in a similar manner, the diameter of the cavity of every water-conducting element was measured by means of an eyepiece micrometer, and recorded. From these observations a number of statistical results were arrived at for each section in the following order: First, an average figure was obtained for the diameters of the elements in question; and secondly, the number of these elements in a unit area was worked out. The results of these calculations were used to determine the proportion per cent. of the area of the wood occupied by the cavities of the vessels and tracheides; and from this percentage figure and the figure for the total area of the wood it was possible to calculate the total area occupied by these cavities in the complete section. The same observations were available also for working out the proportion of wide to narrow elements in the section.

For the purpose of obtaining information as to the variation in the lengths of the vessels it was necessary to apply a method of a different type. It is not possible to observe the lengths of these elements in longitudinal section, for they extend through several centimetres. I had the opportunity, however, of making use of Professor Farmer's apparatus designed for the injection of vessels. The material used for this purpose consisted of fresh stool shoots collected in February. The shoot was cut into a number of suitable lengths for injection, the distances of the several injection surfaces from the base being recorded. After injection, transverse sections were cut at measured distances from each injection surface to determine the maximum distance reached by a specially prepared colloidal solution of Indian ink.

*Accuracy.* Naturally the figures obtained for the diameters and numbers of the elements measured are approximate only. In transverse section the water-conducting elements are very variable in shape, and most of them are by no means circular; the areas were worked out for circles, however, and as far as possible the diameter recorded for each element was that which would give the nearest area with the appropriate formula in view, the measurements being made with a  $\frac{1}{8}$ -inch objective. All the measurements and calculations were made in a uniform way so that the figures obtained should have a comparative value.

*Results.* The data obtained by these means are presented as far as possible graphically, in the form of curves. Taking first the characters shown in transverse section, I have drawn for each shoot a set of graphs to show the change in the constitution of the wood from base to apex. In constructing the graphs the numbers of the internodes were marked along the base line, taking an equal interval for each internode, irrespective of its

length; having taken a special vertical scale for each set of figures, points were plotted for each chosen internode and joined by a curve for each varying character. An examination of these sets of lines will show that they are remarkably concordant for the four shoots examined in this way. In the four cases, nearly corresponding sets of internodes were examined, the interval being shorter towards the apex; the lowest internode cut was the

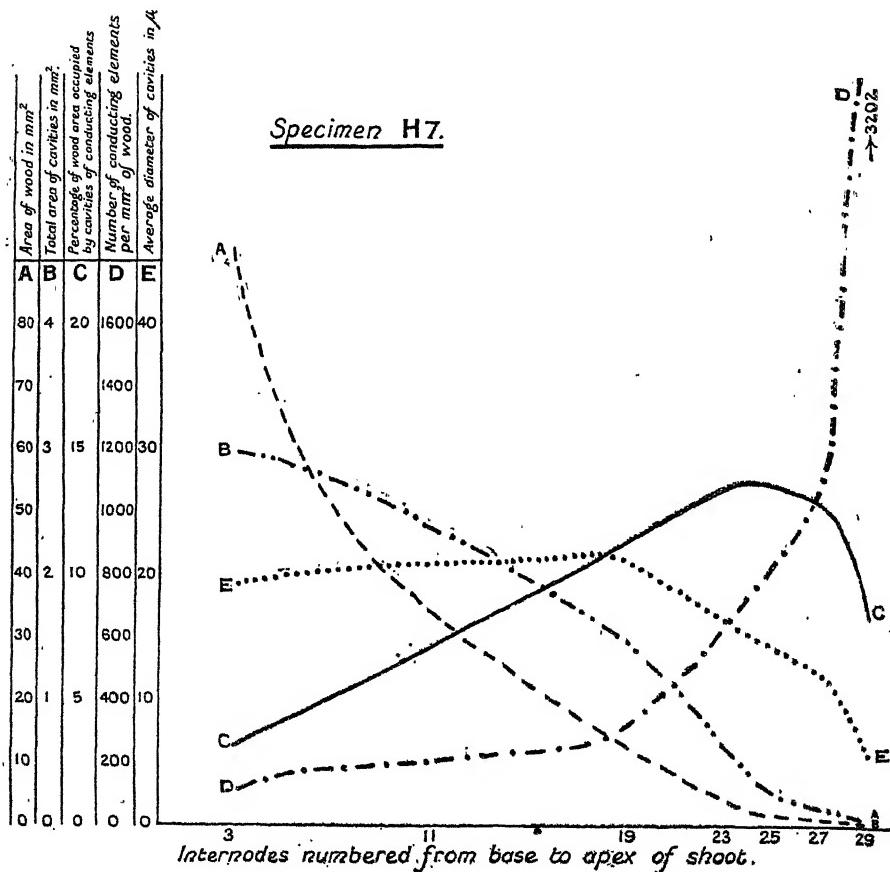
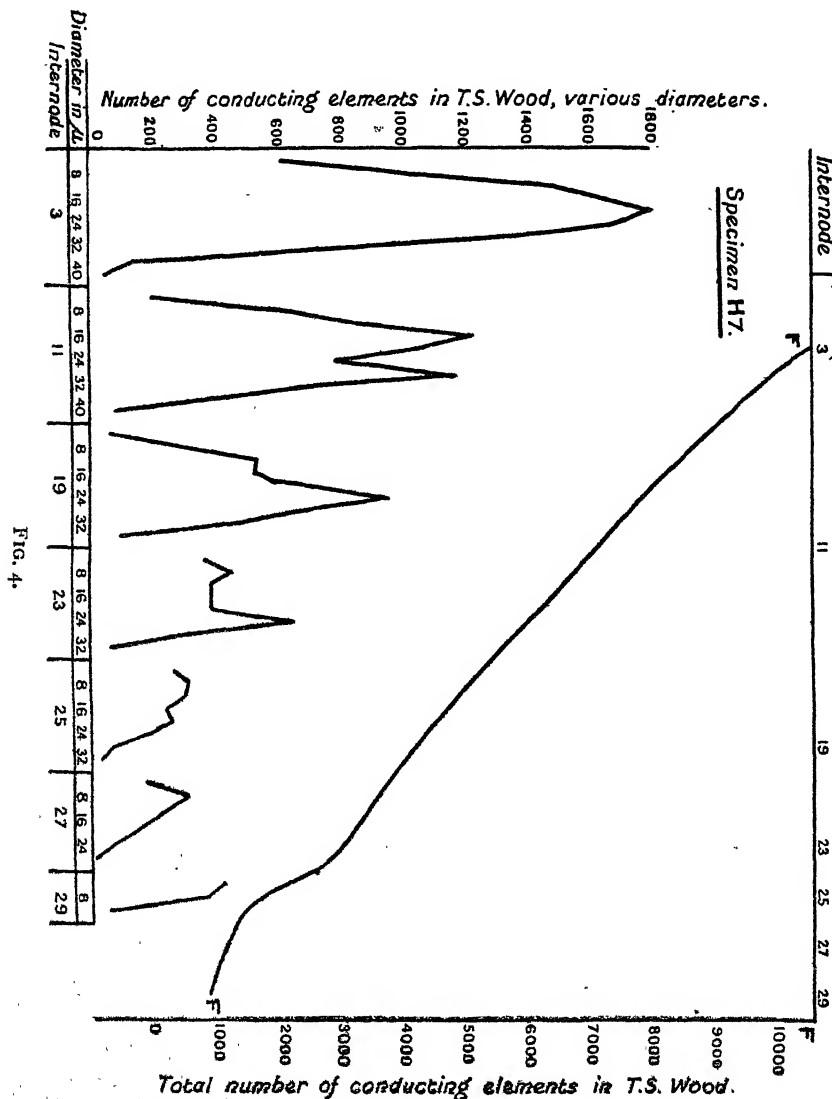


FIG. 3.

third or second, the section being four to five centimetres from the base; the highest internode cut was that immediately below the apex.

*Curve A.* This shows the area of the wood at different levels in the stem, measured in sq. mm. from transverse sections cut at the centres of the selected internodes; it is a smooth curve, indicating a steady decline in the area from base to apex, but falling more steeply at first. The disproportionate amount of wood at the base of the shoots suggests the necessity for extra support in this region, rather than for extra water-

conducting tissue. It will be noticed from Fig. 1 that specimens H<sub>7</sub>, H<sub>8</sub>, and H<sub>10</sub> are all about the same length, although H<sub>8</sub> has fewer nodes. From curve A it is clear that H<sub>7</sub> and H<sub>8</sub> have a greater area of wood in corresponding regions than has H<sub>10</sub>; and that although at the base



H<sub>8</sub> is thinner than H<sub>7</sub>, in the internodes near the apex H<sub>8</sub> has the most wood. H<sub>9</sub> is shorter than the other specimens, and has the least area of wood in corresponding parts. The low figures for the areas of the wood in the final internode are quite close together in the four cases.

*Curve B.* In this curve is shown the total area occupied by the cavities of the water-conducting elements in the complete transverse section of the wood at different levels. The areas are measured in sq. mm., but each unit in the scale for curve B is twenty times as long as the unit in the scale for curve A. As one would expect, this curve, giving a measure of the total conductivity as far as can be obtained in transverse section,

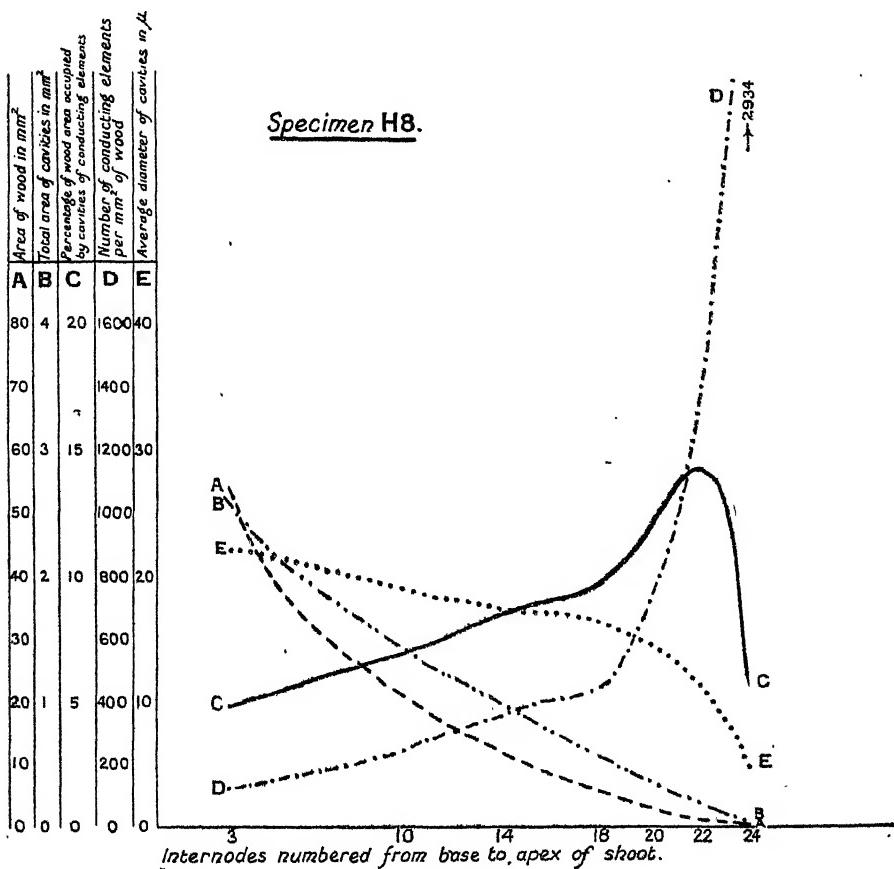
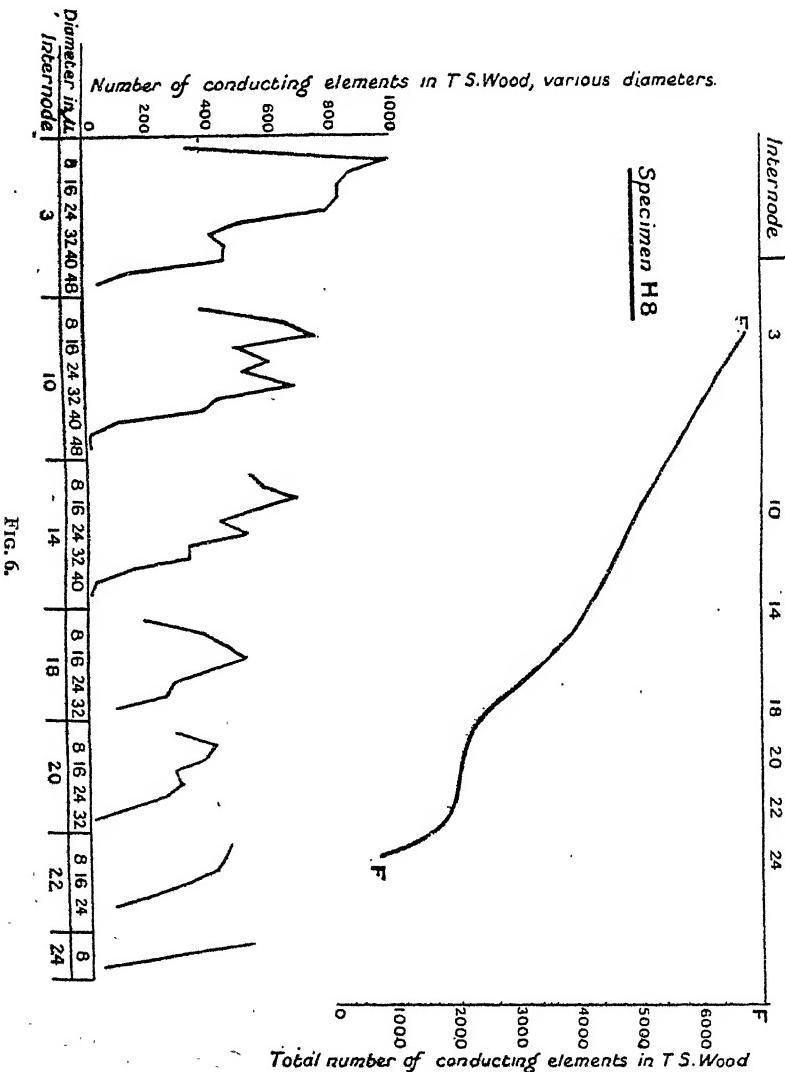


FIG. 5.

shows a gradual decline from base to apex. Compared with curve A, curve B does not fall so steeply at first; this accords with the presence of a greater proportion of mechanical tissue at the base, as suggested above. This is the case especially in H7.

*Curve C* represents the percentage of the total area of the wood which is occupied by the cavities of the water-conducting elements. The figures obtained range between 3.21 and 20.26 per cent. The curve may be taken to indicate, as far as possible in transverse section, the specific conductivity

of the wood at different levels in the shoot. In all four cases it rises from the base upwards for the major part of the stem, and then falls sharply at the end; the maximum percentage is reached at some internode near the apex, and in three cases there is a rapid rise before the maximum,



as well as a rapid fall after. In H<sub>7</sub> the special preponderance of fibres at the base is reflected in the specially low specific conductivity in the lowest internode cut. The low specific conductivity in the final internodes is to be connected, not with a high proportion of fibres, but with the higher proportion of living cells and the small size of the vessels; when the cavity

of an element is very small, its wall occupies a greater proportion of its total area in transverse section. The general rise in the curve up to the maximum is certainly due to the fact that the wood becomes richer and richer in vessels and poorer in fibres, as can be seen clearly from the sections. On the whole, the higher values for specific conductivity, curve C, in H<sub>9</sub> and H<sub>10</sub>, than in H<sub>7</sub> and H<sub>8</sub>, are associated with lower figures for the area

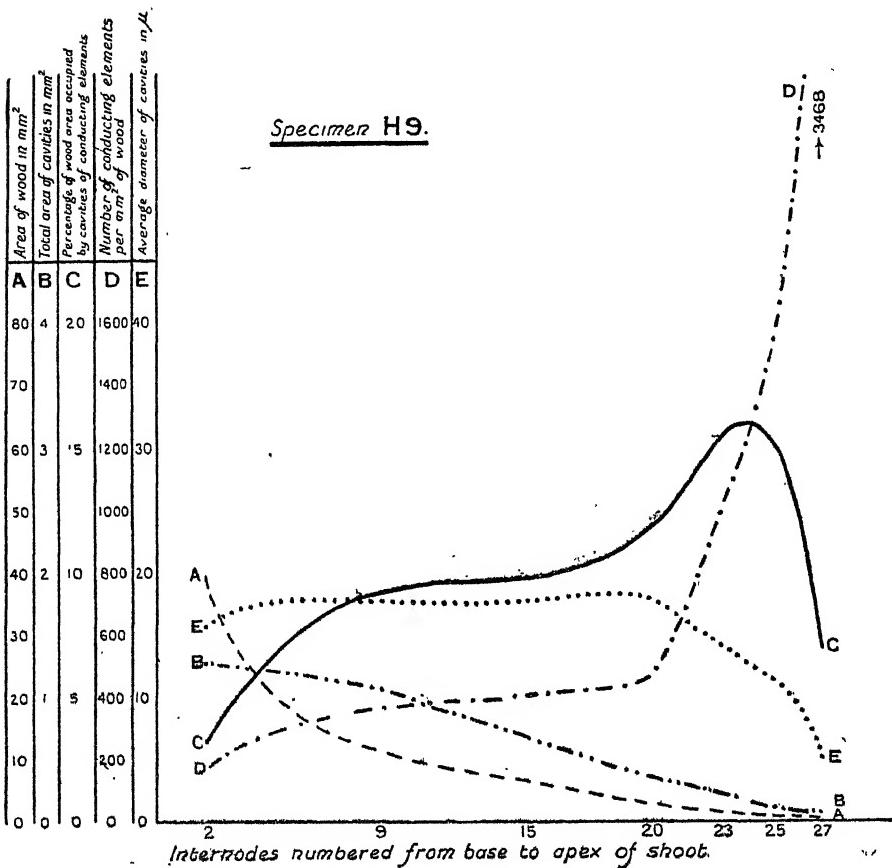


FIG. 7.

of the wood, curve A; this brings the values for total conductivity, curve B, nearer together in the four shoots. It means that there is a greater proportion of fibres in the larger wood sections. The maximum in curve C occurs nearer to the apex in H<sub>8</sub> than in the other shoots; it will be noticed also that at this internode, the third from the top, there is a greater area of wood and a greater value for total conductivity in H<sub>8</sub> than at the corresponding internodes in the other shoots. This may possibly have some connexion with the fact that the terminal bud is present in H<sub>8</sub>.

*Curve D.* The figures for curves B and C were found by counting and measuring the vessels and tracheides in a measured area, and these relations are shown in two more curves. Curve D represents the number of water-conducting elements per sq. mm. in the transverse section of the

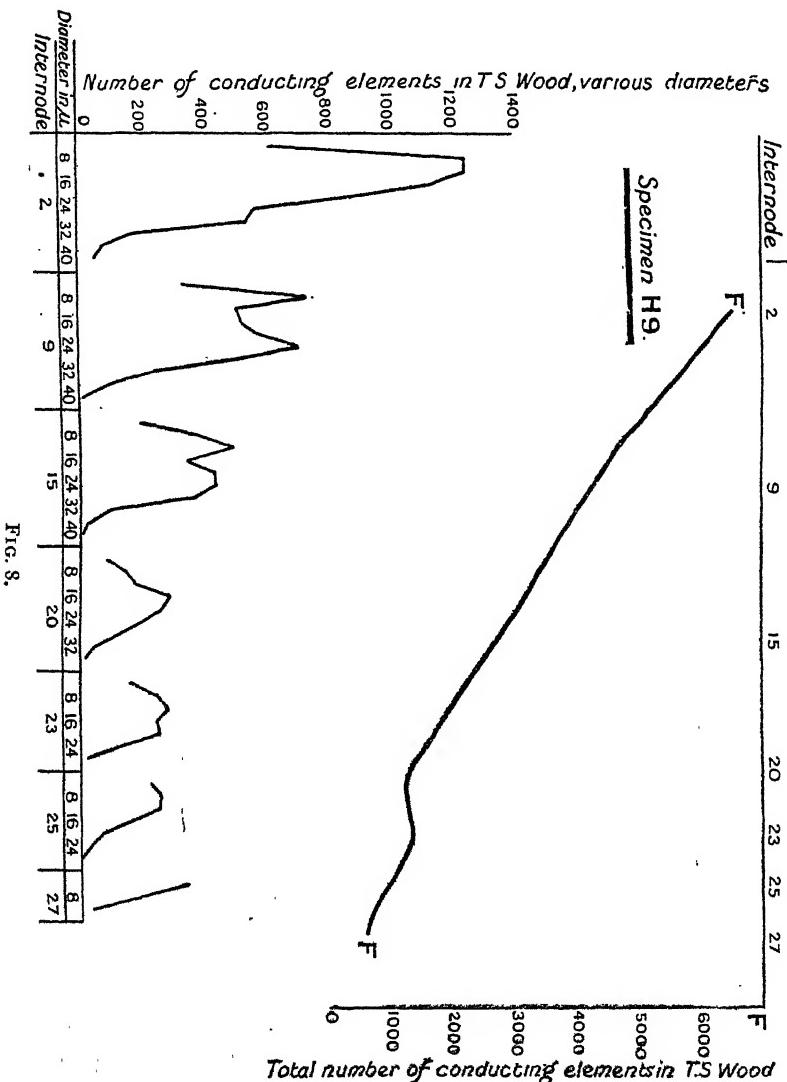


FIG. 8.

wood at different levels in the stem. The figures calculated range from 115 to 4,000 per sq. mm. The curve begins with a gradual rise and ends with a very rapid rise, and it will be seen that the change from the gradual to the steep ascent corresponds with the similar change in curve C. This shows that the rapid increase in specific conductivity in this region is due to

a rapid falling off in the proportion of mechanical elements differentiated, and it may be connected with the smaller size and weight of the leaves borne upon this part of the shoot.

*Curve E* represents the average diameter of the cavities of the water-conducting elements present in the wood at different levels in the shoot. The range of the actual diameters measured was from  $2\mu$  to  $48\mu$ ; and the highest average figure obtained was  $23.27\mu$ . On the whole there is a

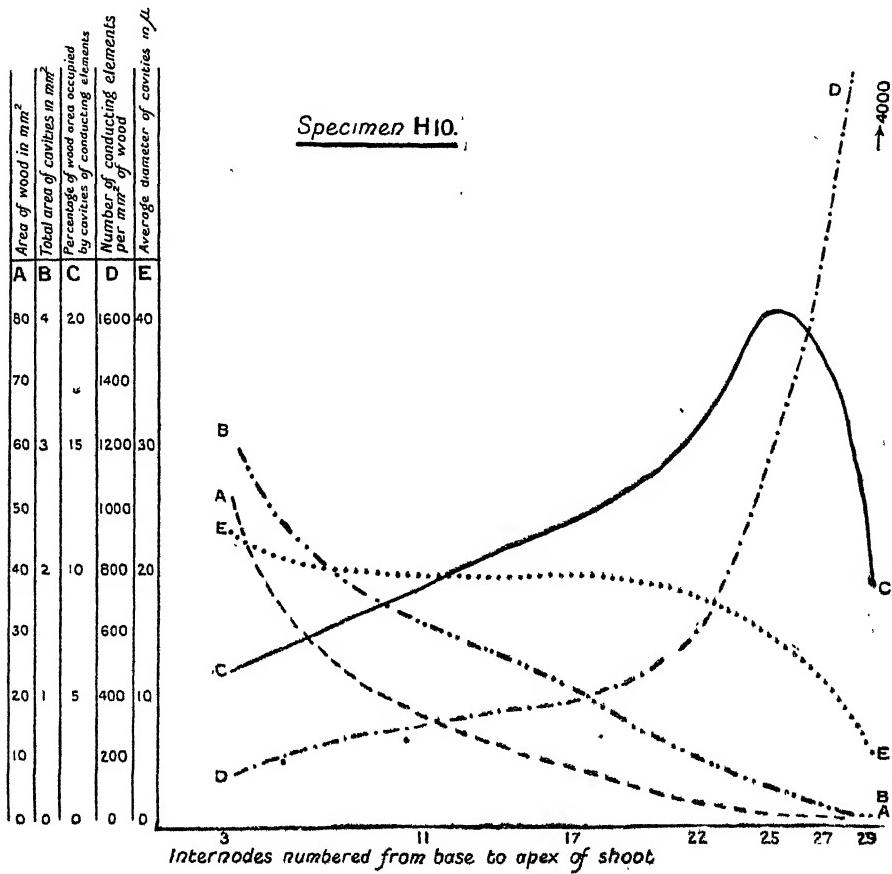
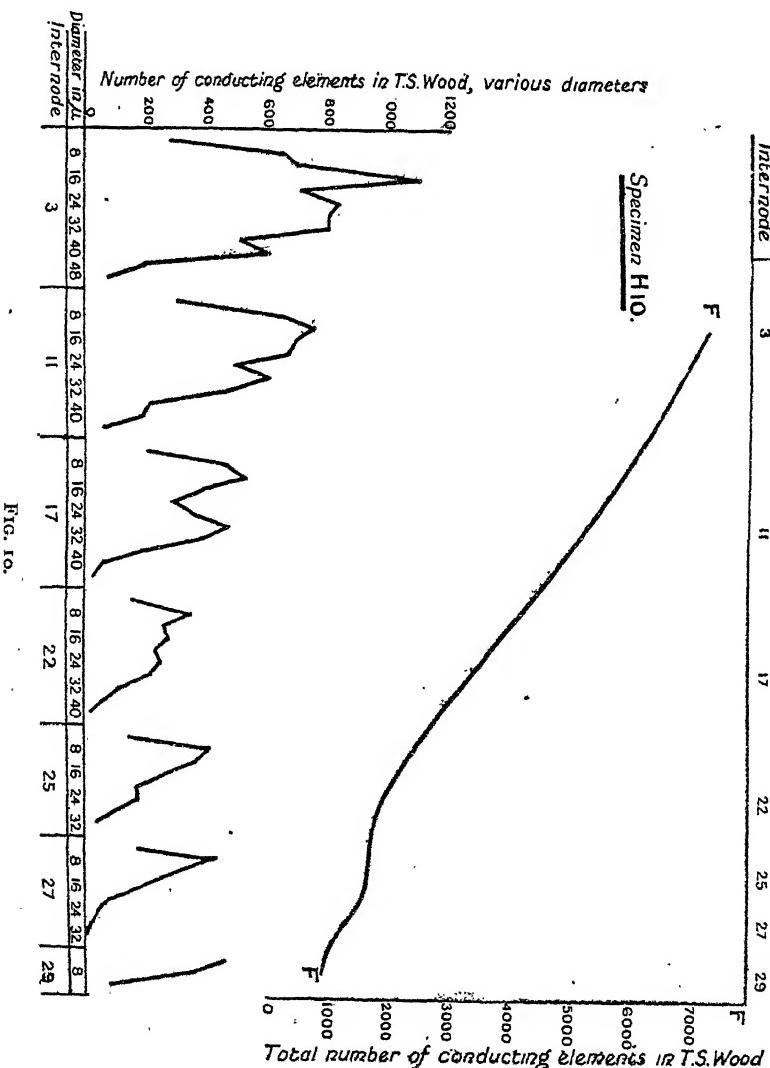


FIG. 9.

general decrease in the size of the vessels from base to apex, but towards the base the proportion of tracheides increases, and, as these are of small diameter, they tend to reduce the average. This is the case especially in the large sections in H<sub>7</sub>, in which the proportion of tracheides is particularly high. The curve keeps fairly level for the lower part of the shoot, and then falls, gradually at first, and more rapidly near the apex. It will be seen that the first bend downwards occurs at the region in the stem

where curves C and D bend upwards, while the second bend in curve E corresponds with the rapid fall in curve C after the maximum point. This shows that the decrease in specific conductivity for the internodes at the top is due chiefly to the second of the reasons suggested above in discussing curve C,



namely, the very much smaller size of the vessels near the apex; for from curve D it is seen that the proportion of vessels to other elements at this region is very large. It must be remembered, however, that the very steep ascent at the end of curve D is itself the result of the fact that all the vessels in this part are very small, and therefore curve C descends with curve E

and does not ascend with curve D. The widest vessels observed were in H<sub>8</sub> and H<sub>10</sub>, in the lowest internodes; the maximum diameters for H<sub>7</sub> and H<sub>9</sub> are smaller. With this absence of very wide vessels may be connected the initial ascent in curve E for H<sub>7</sub> and H<sub>9</sub>, which contrasts with the initial descent in curve E for H<sub>8</sub> and H<sub>10</sub>.

*Curve F* is drawn upon another sheet for each specimen. It indicates the total number of conducting elements estimated for the complete transverse section of the wood at the selected internodes. This curve shows a general decline, with more or less irregularity towards the apex. At the internode corresponding with the maximum in curve C, the wood is seen from curve F to be particularly rich in vessels.

Upon the same sheet is drawn also a set of graphs to show the numbers of conducting elements of different diameters, which make up the total numbers given in curve F; the diameters are given at intervals of  $4\mu$  as they were measured. On the whole, in any one transverse section, the narrow elements are more numerous. The internodes proportionately richest in wide vessels are those towards the middle of the shoot.

Owing to insufficient data, no figures for lengths of vessels are given in this paper, but I have reason to think that such data would not alter materially the conclusions arrived at from transverse characters.

#### CONCLUSION.

In this paper I have described a statistical method of investigating the constitution of wood from the standpoint of its efficiency for conducting water. It is an attempt to record definitely the number, size, and distribution of the elements in the wood which are concerned in this process, and to present the results in a graphical form. The method is intended to serve as a basis for correlating with anatomy the facts of specific conductivity obtained by experiment, and possibly to bring to light further facts in this connexion.

In stool shoots of the hazel the figures have shown a very considerable variation in the constitution of the wood formed during the first season. On the whole there is a general decline in total conductivity and a general rise in specific conductivity from the base of the shoot to its apex. The explanation, in this case, is simple: it has been shown to be related to the provision for the other functions carried out by the wood, and chiefly to the greater proportion of mechanical elements in the lower part of the shoot, providing the support necessary in this region.

I wish to acknowledge in this place my indebtedness to Professor Farmer for the help he has given me in this work, and to thank him for his interest and encouragement.

## ERRATA

In the title and head-lines to pages 569-589

*for* Structural Sigillariostrobus

*read* Structure of Sigillariostrobus

*Annals of Botany, October, 1918*

*face p. 569*

# Mazocarpon or the Structural Sigillariostrobus.

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With Plates XVII and XVIII and four Figures in the Text.

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## PART I. DESCRIPTION OF MAZOCARPON.

### SECTION I. *Introduction.*

SPECIMENS of the megasporangia of this new type have been gradually accumulating for a long time. The large megaspores, showing a considerable range of sectional form and for the most part with a portion of their sporangiæ-wall adhering to their base, are relatively frequent, especially in Shore nodules. Specimens are also in hand from those of Halifax, Stalybridge, Dulesgate, Hough Hill, and Bacup. Two broken but well-characterized sporangiæ of another species<sup>1</sup> have been obtained from a block of the calciferous sandstone material from Pettycur, Scotland.

A transverse section of a cone from an unrecorded locality, and probably specifically distinct, was most kindly contributed by Prof. Weiss from the

<sup>1</sup> Benson: N. Ph. vii, 1908, p. 143, Figs. 25 and 26.

Manchester Collections. I also wish to gratefully acknowledge his loan of an excellent preparation from a Shore ball, from which Fig. 1 is prepared.

In the spring of 1913, having exhausted my own supply of Shore balls in the search for more information of this structure, Prof. Oliver kindly allowed me to cut some of his supply. Almost immediately, by exceptional good fortune, a radial section of the megasporange (Fig. 5) was secured.

This enabled me to correlate a number of other sections. Shortly after, from three successive balls, were added not only a series of five sections tangential to a megasporange but a similar series of four through a well-preserved, large microsporangium. Microsporangia had not previously been observed in the Upper Carboniferous rocks.

Other objects, bearing on this research, found at the same time, will be referred to later.<sup>1</sup>

For the present it is sufficient to add that Dr. Scott, with whom I had hoped to co-operate in the description of the new form, most generously handed over to me his specimens of *Mazocarpon*, including a fine series of three longitudinal sections through a mature megasporangial cone from Hough Hill, and suggested that I should give a short account of the work at the Australian Meeting of the British Association in 1914.<sup>2</sup> For many other tokens of kind interest in the research I owe Dr. Scott grateful acknowledgement.

## SECTION II, *The Megasporange.*

The megasporange resembles in many respects that of *Lepidostrobus*. It is a bulky, radially extended body attached to a bract along its whole length. The wall is composed of a palisade and subjacent parenchyma. The sporogenous tissue is found lying over a well-developed subarche-sporial pad.

The new characters are (1) the large amount of persistent, sterile tissue and its differentiation into various types, e.g. transfusion tissue, tapetal tissue, &c.

(2) The prolongation of the wall into a shovel-shaped distal lamella which fits into the concave upper surface of the bract to which the sporange is attached.

(3) The form and distribution of the spores and their limited number.

It was owing to the persistent, sterile tissue, which by enveloping the dark-coloured spores gives the sporange much the appearance of a sausage-roll, that the name *Mazocarpon* (*μᾶζα* = a loaf) was selected.

A mass of transfusion tissue consisting of scalariform tracheides is clearly to be seen at the base of the pad in the Manchester slide 472 A

<sup>1</sup> See Part II of this paper.

<sup>2</sup> British Association Report, 1914, p. 584.

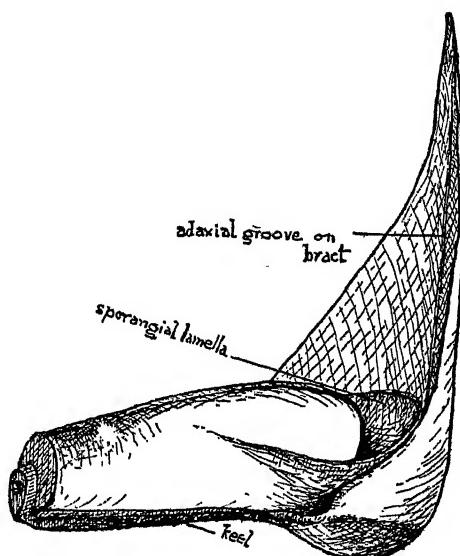
(Fig. 15), and strands of apparently lignified cells would appear to be present in others also, e.g. H. Cn. 519, 4 and 5, but are there cut transversely.

In immature sporangia, e.g. those in the Manchester slide (see Fig. 15), the germinating spores lie in tissue which markedly differs from the pad and certainly suggests barren sporogenous tissue. On this abut the tubular tapetal cells which grow centroskopically into the sporogenous region which they surround. A few of these can be seen in several figures (Fig. 16), but they reach an extraordinary development in an abortive sporangium (Fig. 7) and in the microsporangium (Figs. 13 and 13 a). In the latter the repeated serial divisions of the tapetal cells give a most unusual appearance to the sporangium.

The mature sporangium shown in Fig. 18 measures slightly over 5 mm. in length, and 2·6 mm. in height.

These are approximately the dimensions, as we shall see later, of Dr. Kidston's incrustation specimen of the sporangium of *Sigillariostrobus ciliatus*,<sup>1</sup> with which the new petrifications agree also as respects the size, surface, and probably the form, of the spores. The sporangia occur occasionally in an almost intact condition free from the cone-axis—such are those of Figs. 1, 6, 13, 17, and 18. The abortive specimens, also, which are shown in Figs. 5 and 7, were lying free from any cone-axis. Thus we may regard the sporophyll as liable to fall off even in an immature condition of the gametophyte or 'spore' (Fig. 15). Not only did the sporophyte as a whole fall, but the sporangium seems to have broken up very easily into pieces, each consisting of a 'spore' and a piece of the thick sporangium-wall attached to its base (Fig. 9).

The characteristic formation of a distal lamella, into which the convex base of the bract above must have fitted, was probably a contributory factor, as will be shown later, to the rupture of the sporangium. Distally the lamella springs from the base of the sporangium, but from a higher level at the sides (Text-fig. 1). In the third, or middle, member of a series of five



TEXT-FIG. 1. Explanation in the text. A diagram from a model of the megasporangium of *Mazocarpon*.

<sup>1</sup> See Kidston, loc. cit., Plate II, Figs. 3 and 3 a (or consult Scott's Studies in Fossil Botany, Fig. 96 s, p. 234).

sections tangential to the sporange (H. Cn. 518, 4-8) the lamella is very nearly level with the upper surface of the sporange.

The lamella is shown well in Figs. 6, 5, and 18, and is indicated in others.

Along the dorsal median line of the sporange a ridge is often seen which is doubtless due to the unusual degree of vegetative development the sporange has attained, which has made it capable of accommodating itself to the available space.

In no Upper Carboniferous specimen has the number of spores per sporange exceeded eight.

They will now be described.

### SECTION III. *The 'Megaspore'.*

Owing to their size these can only be examined in section in petrifications. The longest dimension of the spore shown in Fig. 3 is 1.94 mm. One in Fig. 1 is 1.7 mm. across; another in Fig. 1 is 1.52 mm. across.

The circular section in Fig. 9 has a 1.25 mm. diameter.

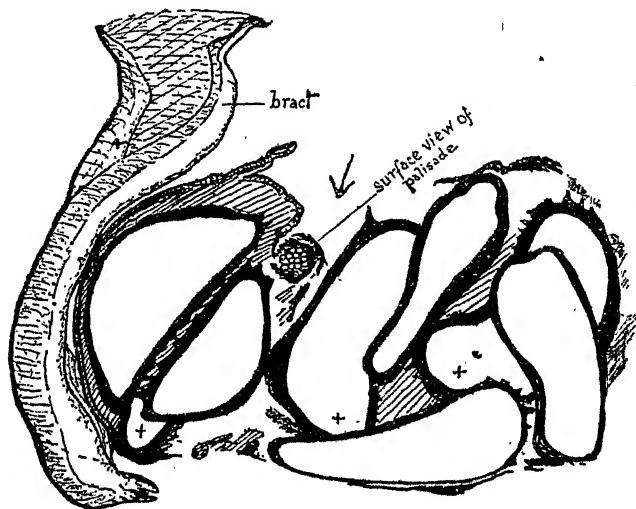
The form is that of a tetrahedral or radially-symmetrical spore which on germinating has taken on lateral development in the restricted space available between the sporange-wall and the cylindrical subarchesporial pad. If we might use a homely illustration we might compare their form to that attained by a limited number of equal pieces of dough disposed over the upper surface and ends of a cylinder, after they had been submitted to such external pressure as would cause them to cover the cylinder without overlapping.

If both the portions of the dough and the distribution of pressure were uniform the form of the pieces would show certain features in common. There would be a tendency to a concavo-convex form, and this would be most marked in those portions which had to 'round' the ends of the cylinder. The problem in *Mazocarpon* is not quite so simple, however, for the sporange narrows rapidly towards the cone-axis and there are occasional instances of the invasion of the pad by the advancing development of the 'spore'.

A remarkable feature in the orientation of the 'spores' is that the organic apex (triradiate scar) abuts directly upon the pad, i.e. is centroscopic with reference to the whole sporange. One can only correlate this fact with the observed character of the pad tissue. This tissue was the chief source of water and food, and one must conjecture that it was a nutritive stimulus which brought about the definite orientation. The megasporangium germinated while still young and, growing in the residual sterile sporogenous tissue, became filled with a prothallial tissue which is often

extraordinarily well preserved (Fig. 4). The venters of the archegonia are to be seen beneath the scar in a large number of cases (Figs. 3 and 18). When the sporange breaks up the 'spore' apex invariably comes away from the pad, but the base of the 'spore' more frequently retains its connexion with the outer tissue of the sporange (see Fig. 9), a fact which may have been of biological advantage, as is the association of the embryo sac with the perisperm or nucellar tissue in the more typical 'seed'.

The spore-wall is obviously not rigid throughout development. Its base is covered with pointed processes which undoubtedly helped it to



TEXT-FIG. 2. A diagram constructed from two successive nearly horizontal sections through a megasporangium on a bract (the keel of the bract is not shown in the section, as it appears in the third section of the series). The spores marked with + were cut in the middle section, and one has been slightly displaced by a stigmariian rootlet entering the sporangium in the direction indicated by the arrow. H. Cn. 525, 2 and 3.

retain its hold on the surrounding tissue. When the prothallus has attained its maximum size the wall appears to have become rigid, as there are several cases of the prothallial tissue being separated from the spore-wall by shrinkage (Fig. 4).<sup>1</sup>

Owing to the permanent tissue uniting the pad to the pedicel of the sporange more spores occur in the upper than in the lower horizontal plane of the sporange. In Text-fig. 2 five spores are cut in the uppermost section (H. Cn. 525, 3), but only three in the lower (H. Cn. 525, 2). The latter three spores are marked with + in the text-figure. As the two planes of section were respectively near the upper and the lower surfaces of the sporange not a single 'spore' has been cut through its organic apex, so

<sup>1</sup> See also Scott's Studies, Fig. 78, p. 188.

that they offer a marked contrast in that respect with the 'spores' in Fig. 18.

If one compares the various figures of 'spores' in Pl. XVII and Text-fig. 2, one sees a wide range of form of sectional area, but one must be on one's guard against assuming that the uncut 'spores' were necessarily so varied in form. As already stated, they probably for the most part agreed in showing a convex base and a plane or concave upper surface. End members in a distal part of the sporangia may have been like the radially cut spore in Fig. 3, i.e. of the shallow crucible form. Such 'spores' when cut tangentially show a circular area, as is shown in Fig. 9. As respects the early stages of development we know but little. An abortive sporangium (Fig. 7) contained some rounded bodies larger than the other elements of the sporogenous tissue, but they give no evidence of a tetrahedral form. The frequent suggestion of the number eight as the maximum of spores per sporangium is consistent with their development from two tetrads, and their origin from a tetrahedral form is indicated by the existence of a triradiate scar (H. Cn. 41 shows this feature in tangential section).

Perhaps the most cogent evidence that the apparently ripe 'spore' always represents a gametophyte is its definite centrosopic orientation. This must have necessitated a considerable alteration of form, which indeed is indicated in the two sporangia of *Mazocarpon Cashii* shown in Figs. 16 and 17.

#### SECTION IV. *The Cone.*

One specimen of a cone was secured from Shore material and cut by Mr. Lomax transversely in series (H. Cn. 527, 1-9).

Another cone from a Hough Hill ball, kindly lent by Dr. Scott, has yielded three longitudinal sections. The sporangia show mature prothallia, and some are cut tangentially, but one exceptionally perfect specimen is cut radially (S. Cn. 1925-7).

There is also a single transverse section of a cone from an unknown locality (? Halifax), M. Cn. 472 A, which has been lent by Prof. Weiss.

The transverse sections of these cones have rendered possible the identification of transverse sections of the peduncle, and denuded cone-axes, which now prove to be no uncommon objects in Coal Measure nodules (e.g. H. Cn. 530, 1-30). These cone-axes have hitherto proved rather enigmatic structures.

From the series 527, 1-9, it is obvious that the cone was pedunculate, and that both the peduncle and the sporophyll-bearing axis were hexagonal in transverse section.

The tangential sections of the cone (S. Cn. 1926) and the transverse sections in H. Cn. 527, and H. Cn. 530, 2-30, clearly demonstrate the arrangement of the cone-scales in a close spiral. The area of attachment of

the cone-scale, i.e. the cone-scale scar or bract scar, measures only one millemetre across. This small area is largely occupied by parichnos strands which accompany the trace and render the attachment very fragile. A great tendency existed for the cone-scales to separate from the cone-axis. Even the relatively perfect sporophyll cut radially (see Fig. 18) had become detached though scarcely displaced.

In the upper part of the cone where the cone-scales are barren or immature they are found attached. These facts point strongly to the conclusion that the sporophylls of *Mazocarpon* were exceptionally caducous. Mr. Lomax informed me, on inquiry soon after the delivery of the specimens, that the sections in the series 527, 1-9, 'may be taken as on an average half an inch apart'. As two of these are in the peduncular region (527, 1 and 2, see Fig. 10) this estimate, if correct, would justify us in regarding the cone as having measured between three and four inches in length. It may have been somewhat longer, as the stele, in the ninth and last available member of the series, is still giving off leaf-traces.

The width of the cone can be obtained approximately by doubling the radial length of the sporophyll and adding the diameter of axis. As the radial section (Fig. 18) measures a little over 5 mm. and the axis (Fig. 20) is 3 mm. we may regard 13 mm. as a little under the width of the cone before it broke up. The upturned and laterally expanded parts of the sporophyll, which appear to have extended to the third in rank above (triple layers being seen in the transverse sections (527, 5 and 7)), must have also added to the thickness of the cone, which we may safely conclude could not have been less than 13.5 mm. in diameter.

*The anatomy of the Cone-axis, &c.* All the tissues are primary. The cortex is differentiated into the usual mechanical outer region and the lacunar middle cortex, which is often fairly well preserved. The stele is circular in section, medullated, and shows projecting protoxylem groups. The sporophyll traces are mesarch and pass out through the outer cortex accompanied by a trough-shaped parichnos strand. The leaf-trace is undivided throughout and travels in the pedicel of the sporangium and not in the keel or dorsal midrib of the sporophyll. It can be detected with difficulty in the distal upturned part of the lamina (see next section).

#### SECTION V. *The Sporophyll or Bract.*

The bract supporting the megasporangium has been met with oftener and in better preservation than that of the microsporangium, which appears in Figs. 12 and 13 as a curiously inadequate structure to support the bulky microsporangium.

The following description is therefore based on the megasporangial bract.

The main features can be recognized from Text-fig. 1, but it is necessary to explain by what means the form of the bract has been arrived at.

Sections are available tangential to the bract throughout the radially extended portion—that is, the series from which Fig. 8 is obtained.

Horizontal sections are available in the transverse sections of two cones and from fallen sporangia (see Figs. 2 and 20).

Sections approximately radial, together with a series of three sections in a plane at an angle of about 45° with the horizontal and radial planes (H. Cn. 530, 14–16), have all been brought into requisition.

In harmony with all these sections we find there was a narrow lamina with a keeled midrib on the horizontal part to which the sporange was attached. In spite of the well-marked keel, the vascular bundle and parichnos did not run in the bract, but, as already stated, in the pedicel or radially extended stalk of the sporange.<sup>1</sup>

The bract thickened considerably just at the distal end of the sporangial attachment, the under surface sinking to form a convex cushion which might be 8 mm. below the plane of the keel. Thus the convex base is not shown in Fig. 2. In this region there was no projecting ridge nor keel.

The free lamina was hood-like in form and measured 6 mm. in the widest part.

As the distal part overlapped the sporophylls above it on the cone we are able to determine that the width increased from 1 mm. to 2 mm., and then to 6 mm., tapering again to a point a little over 6 mm. above the bend.

The erect part had an adaxial groove, so that, as there was no dorsal ridge, the middle line is the thinnest part of the bract. The merest trace of a vascular bundle has been detected near the adaxial surface in the groove.

The whole of the abaxial surface of the bract is strengthened by a layer of thick-walled cells. The wide erect free part of the distal lamina thins out to the margin, but the narrow free part, in the transition region where the bract thickens, has the sclerized layer duplicated like a hem. The upper surface in the concavity (upon which lies the sporangial lamella) is covered with a delicate layer of cells with small lumen under which the tissue usually perishes. Traces of a ligule are seen (H. Cn. 530, 16) in a groove immediately distal to the sporange and beneath its lamella. At this level the bract is still narrow, so that the ligule may be regarded as occupying the distal end of the keel. It may be of interest to point out that the structure of the bract was such that any hygroscopic contraction of the outer surface would have tended to straighten or bend back the structure. If this took place in the cone where the convex surface fitted into the

<sup>1</sup> Dr. Scott has pointed out to me that a similar route of the vascular bundle is shown in *Lepidostrobus Brownii*, as was demonstrated by Zeiller, *Étude sur le Lepidostrobus Brownii*, 1911, and in *Lepidostrobus Fischeri* (now *L. Kentuckiensis*). See Scott and Jeffrey : On Fossil Plants . . . of Kentucky, Trans. Roy. Soc., vol. ccv, 1914, p. 358.

hollow of the sporophyll below in which the sporangial lamella lay, rupture of the sporange and its attachments would necessarily ensue. It has already been pointed out that these were quite unusually fragile.

#### SECTION VI. *The Microsporangiæ.*

In June, 1913, a series of four tangential sections from a microsporangium on a bract, but separate from the cone-axis, was secured from a Shore coal ball (H. Cn. 526, 12-15).

That in 526, 12, is proximal and is injured at the point of attachment to the bract, but shows the tetrads well (Fig. 11).

That in 526, 13, is the most perfect of the series (see Fig. 12).

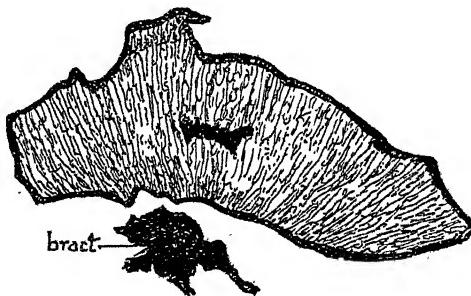
That in 526, 14, is injured very slightly on the dorsal ridge of the sporange, but shows the position in the pedicel of the vascular bundle. On the left is seen a lateral lamella (Fig. 13).

That in 526, 15, is cut through the wedge-shaped sterile distal part of the sporange beyond the attachment to the bract, which is also cut through (see Text-fig. 3).

Though unattached, the body is identified as *Mazocarpon* by its structure throughout, and especially its lateral lamella, which is so thin at the margin that it is turned back on itself. The tissue, which seems to correspond with the tapetal tabular cells of the megasporange, is here divided up into series of short cells.

The spores are formed not merely in one uniform layer over a subarchesporial pad, but in several supplementary masses, with sterile tissue between them, so that the sporange bears some resemblance to the microsporangium of *Isoëtes*. The structure suggests that parenchyma has been formed from potential sporogenous tissue. The spores are still arranged in tetrads, as is so frequently the case in *Lepidostrobus*. They measure 55  $\mu$  by 65  $\mu$  and are thus larger than those of *Lepidostrobus* and smaller than those of *Spencerites*.

There is nothing to indicate how the sporophyll was borne, but 526, 11, has a part of a megasporange and megaspores occurring in the same section as the microsporangium. This, however, is not surprising, as the sections are rich with *Sigillaria* leaves, so that if it can be shown (see Part II) that *Mazocarpon* is *Sigillariostrobus* it would be quite consistent for both kinds



TEXT-FIG. 3. *Mazocarpon* microsporangium. A vertical section through the distal part of the sporangewall where it is free from the bract. H. Cn. 526, 15.

of sporangia to be present even if they were not formed on the same cone. No distal erect part of the lamina of the bract was secured.

### SECTION VII. *Abortive and Semi-abortive Sporangia.*

More or less abortive sporangia have been met with. In one block two were lying at right angles to each other. One was cut tangentially and the other radially (Figs. 5 and 7).

In the upper part of the transversely-cut cone (H. Cn. 527, 1-9) sections pass through cone-scales which are possibly abortive microsporophylls since only megasporangia occur in the fertile part.

In the radial section shown in Fig. 5 the form and length of a normal fertile megasporange has been attained, but only two megaspores have gone on with their development, though the sporogenous tissue is well defined in area. Covering the subarchesporial pad is a palisade to which I have referred as 'tapetal tissue'. A very unusual condition for a full-sized sporange is thus presented to us—a body filled with continuous tissue, embedded in which are two irregularly-shaped 'spores'.

The tangential section of another abortive sporange seen in Fig. 7 shows a much greater development of the 'tapetal' tissue. It occurs above as well as below the sporogenous tissue, and, possibly owing to the arrested development of 'spores', has grown into the sporogenous tissue till it meets or abuts on one or two enlarged cells which show no sign of tetrad formation. It is surprising to see such vigorous growth of the tapetum combined with presumably restricted activity in the sporogenous layer.

The sections through the upper cone-scales in H. Cn. 527, 8 and 9, are interesting because of their form. The vestigial sporange has flattened down upon the bract, and thus the cone-scale is convex above and below, but is provided with a lamellar margin. The contents appear glandular and the vascular bundle takes a median position comparable to that in a foliage leaf. Reference will be made to these later (see p. 585).

### SECTION VIII. *The Problem of Species.*

Three species are recognized: *Mazocarpon Pettycurense*, *Mazocarpon Shoreense*, *Mazocarpon Cashii*.

*Mazocarpon Pettycurense*. Two specimens have been secured from the Calciferous Sandstone Series of Pettycur, Scotland, and are thus of Lower Carboniferous age. These show distinctive characters, although not fully known.

More megaspores are seen in a tangential section of the sporange than have been seen in any similar section in the Upper Carboniferous forms. The microsporange is smaller than the Upper Carboniferous specimen. It is about 4 mm. across, while the latter is 5 mm. across (Figs. 10 and 11). There is a layer of small cells beneath the palisade in the Pettycur micro-

sporange which is sharply defined. In fact we may note that with the exception of this single layer the rest of the persistent sterile tissue is not differentiated into tapetal tissue and parenchyma, but largely partakes of the character of the former.<sup>1</sup> There is as yet no evidence of the existence of a sporangial lamella.

Owing to these distinctive characters, which mark it as a less specialized type, and the great antiquity of these specimens it is desirable to give them specific rank, and the name *Mazocarpon Pettycurensis* is proposed until they are more fully known.

*Mazocarpon Shoreense*. The great bulk of the specimens of *Mazocarpon* described in this paper have been secured from coal balls from many localities in the Yorkshire Lower Coal Measure beds. One cone, the microsporange, and the megasporange from which Text-fig. 2 is taken, were obtained from Shore balls. Those which agree with these specimens I propose to refer to as *Mazocarpon Shoreense*. The diagnosis will be found as a summary of the characters given in the next section (Section IX).

*Mazocarpon Cashii*. Specimens of this species occur in one slide, M. Cn. 472 A, which must be regarded as the type slide. The specimen is given specific rank because of two characters by which it may be distinguished from *M. Shoreense*. The leaf-traces as seen in a transverse section of the cone-axis are surrounded by a sheath as they pass out through the lacunar middle cortex. The transfusion tissue of the base of sporange (see Fig. 15) is more highly differentiated than that observed in *M. Shoreense*.

Other specimens show slight deviations from *M. Shoreense*. Thus the sporange from Dulesgate (Fig. 6) has a wider lamina than would be seen in a tangential section through sporange and keel in *M. Shoreense*, but there are not sufficient data for distinguishing a new species. The sporange of *M. Shoreense* differs so much with its age and place on the cone that a considerable range of variation in detached sporangia must be expected. It is, therefore, inadvisable to multiply species, more especially as it is the aim of the Palaeobotanist to avoid as far as possible leaving a fossil long in a mere form genus such as *Mazocarpon*. If we can show that *Mazocarpon* is *Sigillariostrobus*, the probability is very high that some at least of the specimens of *M. Shoreense* are the fructifications of *Sigillaria mamillaris* with which many of them occur.

#### SECTION IX. Summary of Part I.

*Mazocarpon* is a provisional term used for a form genus of the structural remains of a sporangial apparatus of a Lycopid type.

The cone bore, in a close spiral, cone-scales of the *Lepidostrobus*

<sup>1</sup> See Benson, loc. cit., p. 144, Fig. 25,  $\alpha$  and  $\alpha'$ , where  $\alpha'$  is magnified thirty-nine times and  $\alpha$  only thirty times.

ground-plan, but differing in showing a constriction at the plane of attachment to the axis. The cone-scales are exceptionally caducous.

There is no free lamella directed downwards, but a convex thicker portion without a ridge may extend to about 0·8 mm. below the plane of the keel of the proximal part of the bract (cone-scale). The distal erect part tapers from 6 mm. in width to a point at not less than 6 mm. above. The sporangia are characterized by the possession of much sterile persistent tissue and the proliferation of the distal wall beyond the limit of the attachment of the sporange to the brac

In the megasporange this lamella is shovel-shaped and fits into the adaxial concave surface of the upturned part of the bract.

The megaspores are limited in number (the maximum so far found in any species of Upper Carboniferous age being eight) and germinate *in situ*, while the spore-wall is plastic. The organic apex of each so-called 'spore' is directed centroskopically with reference to the sporange.

There is a considerable range of form, determined by the position which the germinating 'spore' occupies in the space between the sporange-wall and the subarchesporial pad. The form of those occupying the wider distal end of the sporange in *M. Shorensis* tends to be that of a shallow crucible with the organic apex in the hollow, but many asymmetric forms occur. The spore-wall bears pointed prong-like teeth over its convex base, i.e. the surface directed towards the wall of the sporange.

The cone is pedunculate.

The sectional area of cone-axis and peduncle is so far found to be hexagonal.

The cone may be several inches in length and half an inch in diameter. Denuded axes are far commoner than those with cone-scales still attached, only three of which have so far been recorded.

One detached microsporangiophore has been described and is shown to resemble certain incrustation specimens from the same horizon described by Dr. Kidston.

## PART II. THE EVIDENCE FOR THE ATTRIBUTION OF MAZOCARPON TO SIGILLARIA.

### SECTION X. *Introduction to Part II.*

Although *Mazocarpon* has been recognized since 1902, full data were not to hand respecting its form and dimensions. Without sections in recognizable planes or in series, the sporangial lamella was a very puzzling feature, nor was it possible to determine the number of megaspores and their relative position in the sporange. It was in the course of a special search for more material (see Section I) that the association of *Mazocarpon* with Sigillarian leaves was observed. This first clue was obtained in May, 1913, and, being confirmed by Dr. Scott's observations independently, it was

followed up by a thorough examination of the blocks from which *Mazocarpon* had been obtained. Striking association with denuded cone-axes and with the bark of *Sigillaria mamillaris* was found.<sup>1</sup>

More reliable evidence was next obtained by a comparison of the structural material with the incrustation fossils already admitted to be *Sigillariostrobus*. The history of our slowly accumulated knowledge of the cones of *Sigillaria* has been related by Zeiller<sup>2</sup> and Kidston.<sup>3</sup> Goldenberg<sup>4</sup> had previously published diagnoses of Sigillarian cones. Kidston's material, collected by Mr. Hemingway from the Middle Coal Measure Strata of Yorkshire, included for the first time a portion of the cone with sporangia giving some indication of the form, number, and distribution of the megasporangia in a species he named *Sigillariostrobus ciliatus*.<sup>5</sup> Another cone of Lower Coal Measure age with larger sporangia was regarded by Dr. Kidston as bearing microsporangia. The features of resemblance shown by *Mazocarpon* with these specimens are very numerous and will be dealt with in the next section (Section XI).

Incrustations of complete cones were also figured in natural size and cone-axes from which the cone-scales had fallen.

Mr. Hemingway was able to provide further specimens of these denuded cone-axes (R. H. C. Bot. Museum 2, 95), so that material has been available for comparison with surface sections of the *Mazocarpon* cone-axis, and it is found that the scars on each are similar in form and size (set Fig. 19).

Dr. Kidston says (*loc. cit.*, p. 51): 'The shedding of the bracts at maturity seems to be a characteristic of Sigillarian cones and one of the distinguishing points between them and *Lepidostrobus*.' This tendency to fall to pieces is one of the causes in the delay in the interpretation of *Mazocarpon*, but is now shown to be one of the strongest proofs of its Sigillarian nature.

#### SECTION XI. *Detailed comparison of the Structural Material of Mazocarpon with the Incrustation Remains of Sigillariostrobus.*

##### I. *The Megasporange.*

If one compares Fig. 18, which is a single radial section of a megasporange of *Mazocarpon*, or, still better, Text-fig. 2, which is constructed by superposing two tracings of photographs of successive horizontal sections

<sup>1</sup> The bark of *S. mamillaris* was found in such a good state of preservation that not only were the twin bundles of the leaf-trace shown, but the ligule was found *in situ* for the first time (H. Cn. 531, 8). Cf. Arber and Thomas: Ann. Bot., xxiii, p. 514.

<sup>2</sup> Zeiller: Ann. d. Sc. Nat., Bot., 6<sup>e</sup> sér., vol. xix, p. 256, 1884.

<sup>3</sup> Kidston: On the Fossil Flora of the Yorkshire Coal Field (second paper). Trans. Roy. Soc. Ed., vol. xxxix, Part I, 1897.

<sup>4</sup> Goldenberg: Flora Sarapont. foss., Heft I (1855) and Heft II (1857).

<sup>5</sup> For convenience of reference, see Scott's Studies, Fig. 96, A and B; but the original figure more closely corresponds with *Mazocarpon*.

through a megasporange and thus exhibits the total number of spores in the sporange, with Kidston's drawings (reproduced in Scott's Studies, Fig. 96 B), the resemblance is obvious.

It must of course be borne in mind that one is dealing with thin slices of spores in *Mazocarpon*, but with their compressed entire body in Kidston's material.

The dimensions of the sporangia correspond. Text-fig. 2 shows eight spores, and this number is that suggested in *Sigillariostrobus ciliatus*. The size and surface markings of the spores correspond and, so far as sections can be compared with solid bodies, the form is surprisingly similar. In certain cases it can be seen they are distributed peripherally as is shown in *Mazocarpon*, Fig. 18, but there is of course in an incrustation no indication of the actual tissue of the pad, though spaces occur between the spores. The spores in *S. ciliatus* were thought to owe their irregular form to distortion under pressure, and were regarded by Dr. Kidston as probably normally spherical. This interpretation was natural, as some of the bodies show a circular outline. It has been shown, however, in the description of the *Mazocarpon* megaspore that in several cases when a spore is cut tangentially the outline is circular (see Fig. 9). The spores of the French species described by Zeiller appeared to him to be derived from forms 'à peu près sphériques', although he added in a communication he kindly sent me on the subject (May 4, 1914), 'On ne peut, sur des spores aplatis comme on les observe presque toujours, juger avec certitude de la forme primitive'. In *Mazocarpon* the somewhat flattened form is the 'forme primitive'.

It is of course not impossible that some species of *Sigillaria* retained the ancestral form of the megaspore, for the *Mazocarpon* forms described in this paper are undoubtedly due to the germination of the spores while still in the sporange, and we are perhaps hardly justified in calling the body a mere spore.

In *Lepidocarpon* there is evidence that the original form of the spore was tetrahedral, but, with the abortion of three members of the tetrad and the germination *in situ* of the fourth, the whole sporange lumen was eventually occupied. *Mazocarpon* is exactly intermediate between *Lepidostrobus* and *Lepidocarpon*. It allots but one-eighth of the available space to each prothallus, and as these, when mature, are surrounded by a rigid spore-wall with characteristic appendages we incline to call them 'spores'.

If it should be shown that distantly allied species retained the tetrahedral form of the spore it is probable that the number per sporange would be greater, as they would then not have had the space to extend much beyond the normal limit in *Lepidostrobus*.

The spores in Zeiller's<sup>1</sup> restoration of *Sigillariostrobus Tieghemii* in his

<sup>1</sup> Zeiller, loc. cit., Fig. 4.

text-book 'Éléments de Paléobotanique', Fig. 138, are shown as little groups on each cone-scale, which certainly suggest a maximum of eight per sporange, but in an incrustation the sporange-wall has naturally perished, so that we can lay no stress on this specimen.

The fragile nature of the sporange-wall has prevented it being demonstrated in any of Zeiller's and Kidston's specimens, while in *Mazocarpon* no single radial section shows the wall unbroken; the sporange in Text-fig. 2 shows only vestiges of the wall.

The sporange in Fig. 18 is the most complete on the cone in S. Cn. 1595-7, and the wall can be seen to be broken in two places. All the sporangia in the cone are mature, but owing to the broken condition of the sporange-wall it is often difficult to be clear as to the limits of the respective sporangia. This is the usual condition in *Sigillariostrobus* and led one observer<sup>1</sup> to suggest that the spores were formed in the bract without a sporangial wall.

## 2. The Cones.

The characters of agreement between the structural specimens of the cone and the incrustation specimens of the cone of *Sigillariostrobus* both in Zeiller's and Kidston's material extend to the following:

- a. Dimensions.
- b. Phyllotaxy.
- c. Form of bract.
- d. Pedunculate character of the cone.
- e. Agreement between the hexagonal form of section of cone-axis and the dimensions and form of the scar in the incrustations of *Sigillaria mamillaris*.
- f. Cone-scale scar on the cone-axis.
- g. Caducous nature of the cone-scales.

a. *Dimensions.* In Part I, p. 575, we estimated the cone in 527, 1-9, to be between three and four inches in length and a little over 13 mm. or half an inch in diameter. If these measurements are compared with Kidston's figures<sup>2</sup> of *Sigillariostrobus rhombibracteatus*, Plate I, Fig. 3, and Plate II, Fig. 10, they will be seen to correspond very closely with the dimensions of that species.

We cannot of course expect to determine the length of the peduncle, nor is there any reason to consider that all *Sigillaria* cones were of the same size, but the general agreement indicated above in the dimensions of the available material is sufficiently striking.

- b. *Phyllotaxy.* Both show a close spiral phyllotaxy.
- c. *Form of bract.* The description given in Part I, Section V, not

<sup>1</sup> Kidston: 'Les Végétaux houillers recueillis dans le Hainault Belge,' 1911, p. 184, Fig. 32.

<sup>2</sup> Kidston, loc. cit.

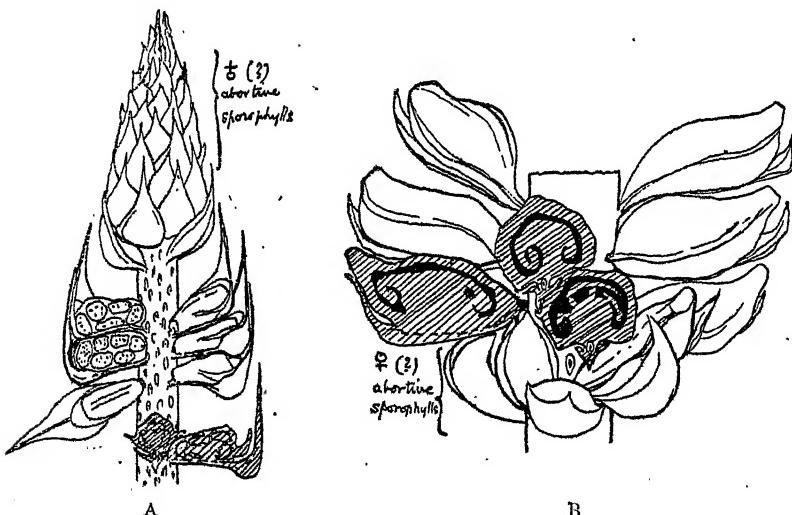
only tallies with the figures given by Zeiller<sup>1</sup> and by Kidston,<sup>2</sup> but it explains the fact of a line like a midrib appearing on the adaxial and not on the abaxial surface. It is shown that in *Mazocarpon* a deep groove occurs on the adaxial surface, but that the abaxial surface is uniformly smooth and shows no rib.

It is unexpected agreement in peculiarities such as this that makes one feel confident one is describing the same thing.

*d and e. Peduncle and its sectional form.* Both *Mazocarpon* and the various species of *Sigillariostrobus* described are pedunculate (Fig. 10). The peduncles, moreover, are deciduous, and Kidston remarks<sup>3</sup> that this seems to be the normal condition in *Sigillaria*. The section area of the peduncle and cone-axis in *Mazocarpon* is hexagonal, and those of *M. Shoreense* have the form and size of the scar which has been attributed to the cone in *Sigillaria mamillaris*.

### 3. The Microsporangia.

It is of especial interest to refer here to a specimen of *Sigillariostrobus* from the Lower Coal Measures of Yorkshire, which has been figured and



TEXT-FIG. 4, A and B. Reconstruction of parts of the mega- and microsporangial cones of *Mazocarpon* (*Sigillaria*) showing the relative size and form of the sporangia. The presence of abortive sporophylls is indicated above the megasporophylls in A, and below the microsporophylls in B. Some of the sporophylls on each cone are represented in section, the cut surface being hatched. Both structural and incrustation material has been utilized in this diagram. For complete incrustations of cones of *Sigillaria* the works of Zeiller and Kidston should be consulted. A represents the upper part of a megasporangial cone; B represents the lower part of a microsporangial cone.

described by Kidston<sup>4</sup> in his above-mentioned work. The cone is of the same geological age as *Mazocarpon*. The sporangia, regarded by Kidston

<sup>1</sup> Zeiller, loc. cit., Fig. 5, Plate XII.

<sup>2</sup> Kidston, loc. cit., p. 56.

<sup>3</sup> Kidston, loc. cit.

<sup>4</sup> Kidston, loc. cit., Plate II, Fig. 1.

as microsporangia, are split away from the cone-axis, but are still in the same relative position in which they were when attached. The sporange seen laterally (*d'*) is very like the microsporangia of *Mazocarpon*. As to the dimensions, the large bodies (*b*, *c*, and *e* in the Fig. 1) given by Kidston<sup>1</sup> agree in every particular with that of the microsporangia of *Mazocarpon*, which was nearly 5 mm. across (Figs. 10 and 11) and formed a lateral lamella.

The lower cone-scales which show unusual spore contents are possibly abortive megasporangia at the lower part of the cone and are analogous with the abortive (?) microsporangia at the apex of the megasporangial cone in *Mazocarpon* (H. Cn. 527; 8 and 9).

This remarkable specimen so happily interpreted by Kidston goes far to indicate that the fertile mega- and microsporangia of *Sigillaria* were at least sometimes borne on different cones, but showed vestiges, respectively above or below, of the cone-scales of the other type (cf. Text-fig. 4). This agreement with a very difficult specimen strengthens the evidence that *Mazocarpon* is *Sigillaria*, since the exceptionally bulky microsporangia of *Mazocarpon* agrees in both form and size with specimens already regarded as microsporangia of *Sigillariostrobus* by Dr. Kidston.

### PART III. GENERAL DISCUSSION OF RESULTS.

- a. Biological aspects of the new structure.
- b. Possible bearing of the work on the affinity of *Pleuromoia* and *Isoëtes* and of genera of Lepidodendraceac.
- c. General conclusions.

### SECTION XII.

#### a. Biological aspects of the new structure.

A few words might be devoted to the biological aspect of the *Mazocarpon* type of megasporange. The presence in some sporangia of ruminating 'spores' which lie in a relatively large mass of sporogenous tissue (Fig. 15) would seem to indicate that the sporange is derived from an ancestral form with a larger number of functional spores. The decrease in the number appears to have been correlated with a limited special increase in the size of the prothallus, for when only one is developed in a sporange it has only attained the normal size (Fig. 5) found in an eight-spored sporange. The seed habit is approached in two ways: (*a*) the megaspores germinate within the sporange and (*b*) the sporange undergoes a certain amount of vegetative development. Fertilization is, however, impossible until fragmentation of the sporange has taken place, owing to the centroskopically directed archegonia. Thus a new factor is introduced and the lamella

<sup>1</sup> Kidston, loc. cit., Plate II, Fig. 1.

seems to have been framed so as to act as the fulcrum when the bract above straightens. Each prothallus would normally secure, by means of its toothed wall, a portion of the sterile sporangial (nucellar) tissue when the sporange broke up, and thus theoretically we find eight seed-like bodies produced from one sporange. Both the type and the adjustments are new to botanical literature.

b. *Possible bearing of the facts ascertained on the problem of the Affinity of the Pleuromoia and Isoëtes, and of genera of Lepidodendreae.*

Throughout the discussion on the incrustation specimens of *Sigillariostrobos*, whether by Goldenberg, Zeiller, or Kidston, mention has been made of the resemblance the genus *Sigillaria* shows to *Isoëtes*.

It may be worth while to inquire whether our new knowledge of the structure of the cones we must now attribute to *Sigillaria* gives us any corroboration of this view.

Potonié summarized<sup>1</sup> the resemblances as respects vegetative organs and accepts *Pleuromoia* as an intermediate form occurring rather happily in an intermediate epoch.

The character in which the sporangia of *Isoëtes* agree with *Mazocarpon*, besides the Lepidostroboid characters of radial extension, &c., is the persistence and relative abundance of sterile tissue. *Isoëtes* shows trabeculae in both mega- and microsporangia which may be suggestively compared with the large trabeculae of the *Mazocarpon* microsporangiæ (Fig. 13). It is possible also that the *Mazocarpon* type of sporangiæ may afford some clue to the better interpretation of the unusual features of the cone of the Mesozoic incrustation, *Pleuromoia*. The wedge-shaped distal part (Text-fig. 3) of the *Mazocarpon* microsporangiæ is not unlike the projecting (i. e. distal) part of the sporangiæ in *Pleuromoia*, as seen in Potonié's Fig. 453 B in the above-mentioned treatise. Moreover, some of the apparent irregularity of the cone of *Pleuromoia* may be due to the difficulty of distinguishing the wedge-like expansion of the sporangiæ from the bract which appears similar to it in outline.

It is even possible that such an expansion may, in an incrustation, have adhered to the bract above and thus given rise to an appearance of a sporangiæ growing on the lower surface; but the figures given by Solms-Laubach<sup>2</sup> are more suggestive of the sporangiæ being partly inserted in a fovea, as in *Isoëtes*. In fact the regular striations of the *Pleuromoia* sporangiæ are more reminiscent of the trabeculae of *Isoëtes* than of the bulky irregular trabeculae of *Mazocarpon*. From these considerations it becomes possible to accept freely the suggestion of affinity between the genera if judged solely on the characters of their vegetative organs. Prof. Lang's

<sup>1</sup> Engler: Pflanzenfamilien, Teil I, Abt. 4, p. 752.

<sup>2</sup> Solms-Laubach: Bot. Zeit., Heft xii, 1899, p. 227, Fig. 7, Taf. VIII.

recent contribution<sup>1</sup> to the anatomy of *Isoëtes* does not essentially affect the problem. With reference to the relationship of *Sigillaria* to the other Lepidodendreae it might be useful to point out that the anatomy of the cone of *Mazocarpon* tends to prevent any great phylogenetic significance being attached to the presence of a double leaf-trace in some species of *Sigillaria*. *Mazocarpon* has but a single trace in its cone-scale, although there can be but little doubt that some of the Shore specimens belonged to *Sigillaria mamillaris*, as that is the species which was prevalent in the Shore balls yielding the bulk of the material, and *S. mamillaris* is one of the two species of *Sigillaria* in which the double leaf-trace has been identified. It is a well-recognized fact in morphology that the cone-scale is likely to retain ancestral characters longer than the foliage leaf, so that the division of the leaf-trace may be regarded as a recent character.

Again, we may ask if the more detailed knowledge of the sporangia throws any new light on the interrelationships of the genera of Lepidodendraceae. It has contributed to the removal of the view that was held by some botanists<sup>2</sup> that the sporange of *Sigillaria* had relatively lessr adial extension than that of *Lepidodendron*. Bearing in mind the distal extension of the sporange and the large mass of sporogenous tissue which is only partially fertile, we find no grounds for rejecting the very cogent theory that *Sigillaria* was an offshoot from some early Lepidodendroid stock. Though comparatively rare in the Lower Carboniferous rocks, a relatively unspecialized type of *Mazocarpon* (*M. Pettycurens*) was already evolved, and with very little change the type persists to the Upper Coal Measures, where it is recognizable as incrustations. It is increasingly probable that it survived as *Pleuromoya* in the Mesozoic and as *Isoëtes* to the present day.

### c. General Conclusion.

In conclusion we may point out that when Zeiller made his discovery of the characteristic leaf-scars in vertical series on the peduncle of *Sigillariostrobus Tieghemii*, in 1884, he really laid the foundation for the diagnosis of *Mazocarpon* as *Sigillaria*. As Dr. Scott says:<sup>3</sup> 'This correlation having once been effected it became possible to identify various other specimens as cogeneric, and the genus *Sigillariostrobus* now includes several species.' Among these species, those of the structural remains, *Mazocarpon*, as now described, may well claim a place. Moreover, if we take into account that we can trace *Mazocarpon* from the Coal Measures back to a period when *Sigillaria* was comparatively rare it is even probable that it is the characteristic type of sporangial fructification of the whole genus.

<sup>1</sup> Lang: Studies in the Morphology of *Isoëtes*, I and II. Mem. and Proc. of the Manchester Lit. and Phil. Soc., vol. lix, Part II, 1915.

<sup>2</sup> Lady Isabel Browne: N. Ph., vi, pp. 153-6.

<sup>3</sup> Scott: Studies in Fossil Botany, p. 232.

## DESCRIPTION OF PLATES XVII–XVIII.

Illustrating Professor Margaret J. Benson's paper on *Mazocarpon* or the Structural *Sigillariostrobus*.

(All the figures are micrographs.)

H. = R. H. C. Collection.

M. = Manchester , ,

S. = Dr. Scott's , ,

*a.* = archegonium; *b.* = bract; *b.l.* = adaxial groove on distal part of the bract; *b.s.* = bract scar; *d.l.* = distal lamella of the megasporange; *s.* = spore; *s.t.* = sporogenous tissue; *t.* = tapetum; *tr.* = transfusion tissue; *v.b.* = vascular bundle.

## PLATE XVII.

Fig. 1. Obliquely cut megasporange showing four to five megaspores and the persistent parenchyma of wall and subarchesporial pad. M. Cn. R. 758; Shore.  $\times 12$ .

Fig. 2. Horizontal section of the bract showing the distal part and the narrow keel beneath the pedicel of the sporangium. H. Cn. 37; Dulesgate.  $\times 30$ .

Fig. 3. A nearly radial section of a megasporange from the same cone as Fig. 18. The venters of two archegonia, *a.*, are clearly shown in the prothallus. The spore-wall shows the characteristic dentate apiculi. S. Cn. 1927.  $\times 47$ .

Fig. 4. Approximately radial section through a spore with the prothallial tissue well preserved. The ring at the base is not an archegonium. H. Cn. 41; Dulesgate.  $\times 25$ .

Fig. 5. A nearly radial section of a partially abortive megasporange showing only two spores. The distal lamella, *d.l.*, looks in section like a rhinoceros tusk. H. Cn. 519, 4; Shore.  $\times 12$ .

Fig. 6. Tangentially-cut megasporange on bract near distal end of sporogenous region. The internal tissue has perished. The relation of the lateral parts of the sporangial lamella to the wide bract is shown. H. Cn. 22; Dulesgate.  $\times$  about 20.

Fig. 7. A tangential section of an abortive sporangium showing the extraordinary development of tapetal tissue and a few rounded cells which suggest (?) abortive spores. They are not in tetrad. The vascular bundle, *v.b.*, in the pedicel is well shown. H. Cn. 519, 6; Shore.  $\times 22$ .

Fig. 8. Tangential section of a megasporange attached to a bract. It is one of a series of five sections through the sporangium. It shows the vascular bundle, *v.b.*, in the pedicel and the parichnos. The character of the pedicel explains the easy detachment of the sporangium from the bract. H. Cn. 518, 7; Shore.  $\times 24$ .

Fig. 9. Oblique section through the distal end of a sporangium cutting a megasporange tangentially. The spore shows in this plane a circular sectional area. This section explains the occasionally circular form of megaspores in incrusted specimens. S. Cn. 1546; Halifax.  $\times 26$ .

Fig. 10. A transverse section of the peduncle of a cone shown in Fig. 20 and described in the text. H. Cn. 527, 2; Shore.  $\times 13$ .

Fig. 11. A high-power photograph of the spore-bearing region and upper wall of a microsporangium. The spores are grouped in tetrad. H. Cn. 526, 12.  $\times$  about 100.

Figs. 12 and 13. The two median members of a series of four tangential sections through a microsporangium (the fourth or most distal in the series is shown in Text-fig. 3). The relative size of sporangium and bract is very striking; also the large amount of sterile tissue in the sporangium and the approximation in Fig. 13 to the construction of trabeculae, as in *Isoetes*. There is no sharp distinction, as in the megasporangium, between the subarchesporial tissue and tapetum (cf. Fig. 7). H. Cn. 526, 13 and 14; Shore.  $\times 23$ .

Fig. 13 a. A part of section 13 showing the details of the tapetum. *v.b.* indicates the position of the vascular bundle. As Fig. 13.

Fig. 14. An obliquely-cut section of a sporangium showing the lamella exactly fitting into the concavity of the bract. H. Cn. 35; Dulesgate.  $\times 9$ .

PLATE XVIII.

Fig. 15. *Mazocarpon Cashii*. Part of the transverse section of a cone from the axis of which the sporangia of Fig. 17 had become detached. The hexagonal form of the axis and the details of the leaf-traces are well shown. M. Cn. 472 A.  $\times 25$ .

Fig. 16. *M. Cashii*. Obliquely tangential section through a sporange in which the germinating spores have not yet reached their final form. The spore on the right can be seen to have already established its centrosopic position, for its organic apex is directed towards the tapetal covering of the subarchesporial pad. The space occupied by the sporogenous tissue, s.t., would eventually have been occupied by the convex base of the 'spore', as in Figs. 1 and 18. A large mass of transfusion tissue is seen at tr. As Fig. 15.

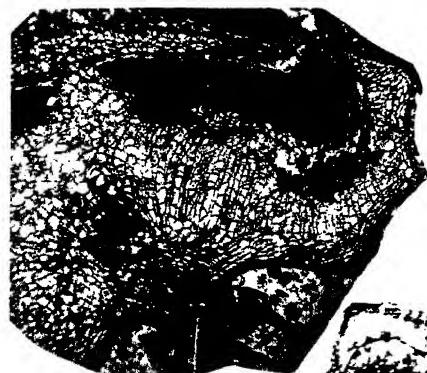
Fig. 17. A low-power photograph of part of the fragmented cone, parts of which are shown in the two previous figures. The section passes through several overlapping bracts, b., one of which shows the adaxial groove, b.g. As Fig. 15.  $\times 12$ .

Fig. 18. Part of the middle section of a series of three through a fine megasporangial cone. It shows a radial section through a sporange on its bract, b., and the bract, b', of the sporange above fitting into the groove between the distal lamella, d.l., and the fertile part of the sporange. Unfortunately the coal ball had been cut away too close to the cone, so that the distal ends of the lamella and bract are incomplete. Six spores can be counted in this section of a sporange, three of which are cut radially through the organic apex. The spore on the right shows prothallial tissue and an archegonium, a., but the prothallus has been invaded by a stigmarian rootlet. In the original the vascular bundle, v.b., can be detected in the pedicel. S. Cn. 1926; Hough Hill; Stalybridge.  $\times 15$ .

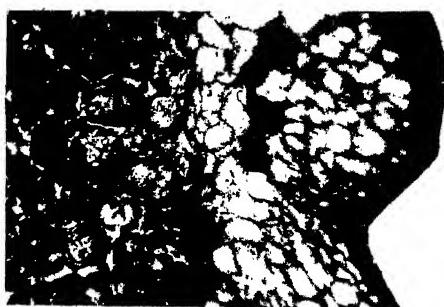
Fig. 19. A surface section of the axis of the above cone showing the form and small size of the cone-scale scars (bract scars), b.s. S. Cn. 1925; Hough Hill.  $\times 15$ .

Fig. 20. A transverse section of the upper part of another megasporangial cone. The hexagonal form of the axis and inner cortex is well shown, while the stele is circular in section. Note also the constriction of the bases of the bracts, which is a Sigillarian character. H. Cn. 527, 7; Shore.  $\times 13$ .

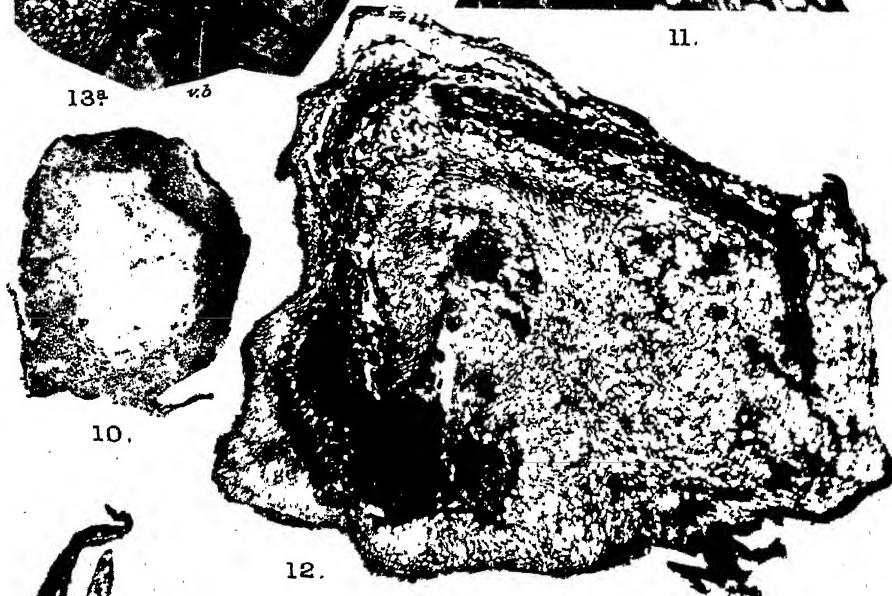




10. n. 8



11.

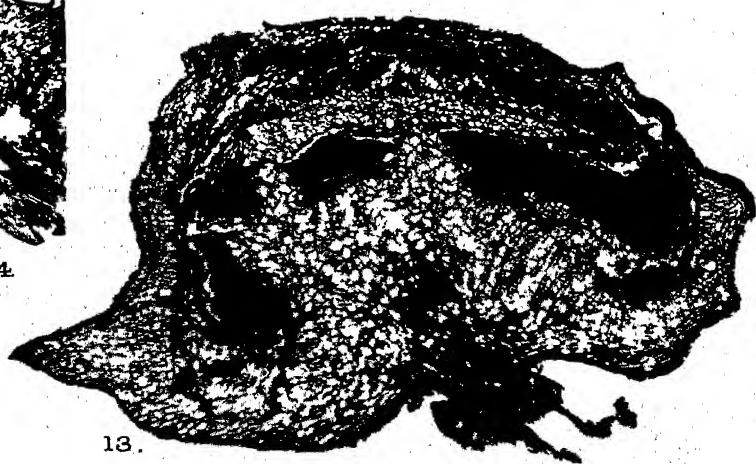


10.

12.

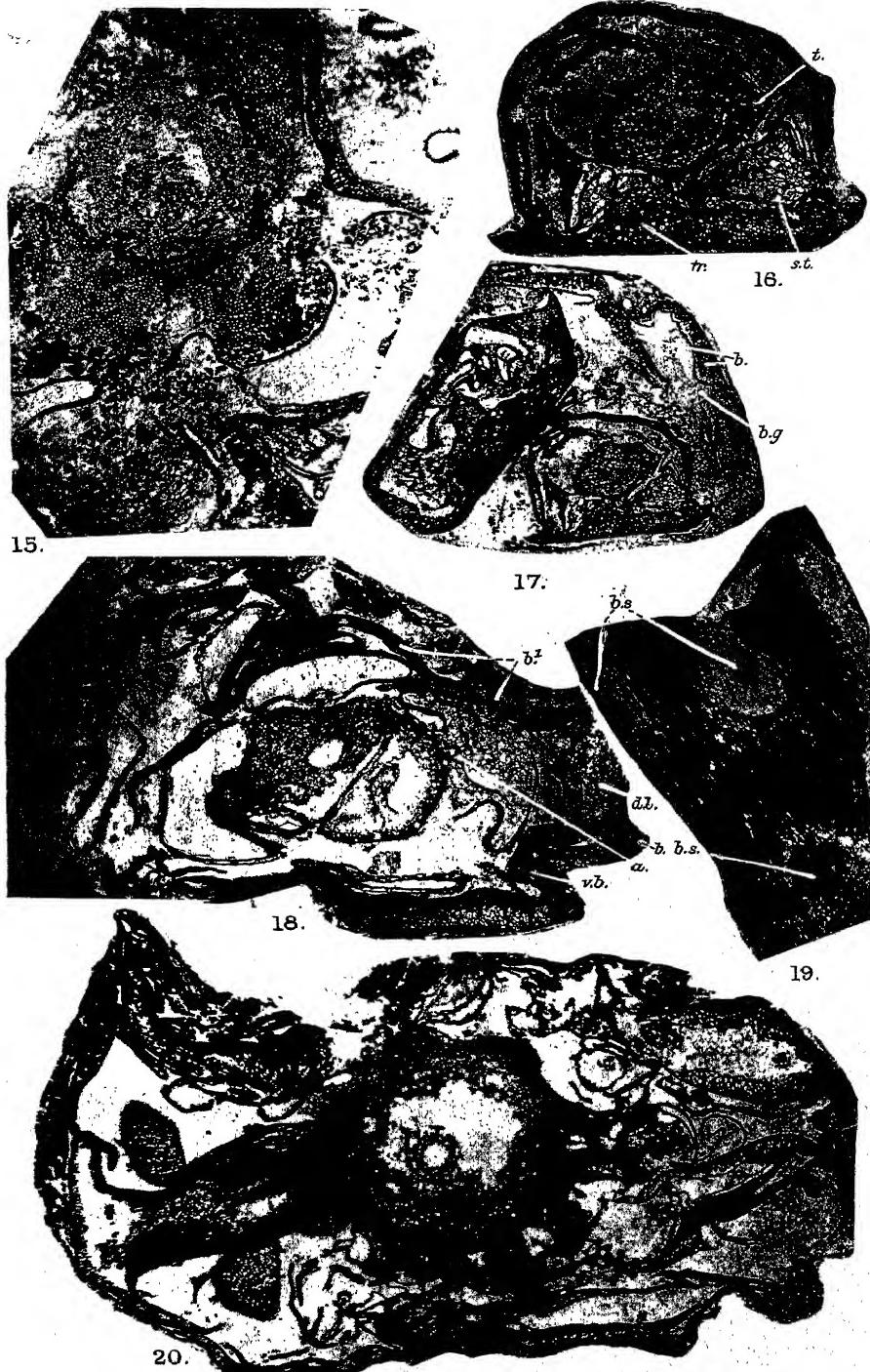


14.



13.







# The Influence of Immersion in Certain Electrolytic Solutions upon Permeability of Plant Cells.

BY

MAUD WILLIAMS, B.Sc.

With two Figures in the Text.

MUCH recent work upon the cells of plants shows the permeability of the cell protoplasmic membrane to undergo modification during immersion in certain solutions. Such immersion may cause the exit of constituents of the sap which are normally unable to penetrate the membrane, or it may render possible the entrance of materials from outside the cell, which are unable to pass into the cell in the ordinary course.

Czapek, as a result of his study of the influence of ethers, alcohols, &c. (1), comes to the conclusion that surface tension of the liquid surrounding the material is the chief factor in producing these abnormal permeabilities. According to this writer 'all substances of the most different chemical character began to injure the cell just when the surface tension had reached the critical value' (2). This critical surface tension is stated to be 'about two-thirds relatively to that of water'. On the other hand, there are numerous experiments which show that the cell-membrane may be rendered permeable to the electrolytes contained in the vacuole by immersion in electrolytic solutions of which the surface tensions are far removed from Czapek's critical value. Thus Miss Hind records abnormal permeability produced by immersion in very dilute acids (3), while Stiles and Jørgensen obtain similar results for many solutions, including magnesium and sodium chlorides (4).

Since surface tension considerations alone cannot explain the alterations of permeability other possibilities must be considered, and it is of interest to find how far the time of immersion in a particular solution affects the question.

An endeavour has here been made to deal throughout with one type of plant cell and

- (a) to find what solutions are capable of rendering the protoplasm permeable to one particular material to which it is normally impermeable;

- (b) to find if there be any connexion between the concentration of the solution in which the cells are soaked and the time of immersion necessary to affect the protoplasm.

#### METHODS AND MATERIAL.

It was decided to deal with cells containing tannin, and to use ferric chloride as the material to which the membrane was to be rendered permeable.

In many plants cells occur which are rich in tannin. This substance occurs, in such cases, in solution in the cell sap, and in the particular plant used is usually insufficient by itself to produce coloration of the cell. The protoplasm of the healthy cell is impermeable to the tannin and, for very long periods at least, it is impermeable to ferric chloride solution in the strength used (0.2 gramme per 100 c.c.).

As a consequence, sections of the plant can be soaked for a matter of two hours in the ferric chloride without a blue coloration appearing in the cell, because the tannin and iron solution cannot come in contact. When the membrane has been altered, however, soaking for three minutes in the ferric chloride will produce a clear, easily recognizable blue colour in the cell.

In order, as far as possible, to equalize diffusion effects, sections must be examined after a fixed time in the iron solution. The short interval above mentioned was chosen in order that the influence of the ferric chloride itself upon permeability, if any, should be negligible.

The cells chosen were situated in surface strips taken from the leaf-stalks of London Pride (*Saxifraga umbrosa*). Under the upper epidermis the tannin-containing cells occur in chains, separated by tracts of cells without tannin. The cells are large, rectangular ones, which are easy to study, and the tissue is in good condition for the greater part of the year. The particular plants used were all grown upon the same kind of soil, and all strips used, except in preliminary experiments, were washed in a large quantity of distilled water for twenty-four hours to remove tannin from the torn cells, before immersion in the electrolyte chosen.

Before one could judge whether the abnormal permeability was general in any test, it was necessary to know approximately what proportion of the cells probably contained tannin in any section. To ascertain this point preliminary experiments were made with strips treated with 1 per cent. caffeine solution. This liquid readily penetrates the cell membrane and unites with the tannin to give a heavy precipitate, which causes the cell to darken.

A number of these experiments were made on various dates, and the cells with, and without, the precipitate were counted. No sections showed fewer than 45 per cent. of the cells to contain tannin; the usual result was

about 50 per cent., while in a few cases as high as 64 per cent. was reached.

In the experiments with the electrolytic solutions, bottles holding 30 c.c. of the liquid chosen were used for the immersion of each five or six sections. Sections were removed after known times, soaked in the ferric chloride, transferred to a slide and mounted in more of the ferric chloride under a large cover-slip, and examined after three minutes altogether in contact with the iron salt. The change of permeability was considered complete if traces of blue could be seen appearing in about half the cells of the section excluding marginal cells. These latter were excluded because it was possible they might have been injured in the preparation of the material. The colour observed rapidly deepened during the next few minutes. The low power had therefore to be used to consider a section as a whole; the high power was afterwards used on individual cells to study the appearance of the protoplasm.

Since such methods are open to many sources of error no great degree of accuracy was aimed at in preparing the electrolytic solutions. The ordinary 'pure' chemicals of a laboratory were used, and a solution of each made up. Small quantities were withdrawn and diluted as required for the separate tests. The ferric chloride was made up freshly every few days.

To accompany the experiments upon each concentration sections from the same part of the plant as those being immersed in the salt solution were left in distilled water, and examined from time to time.

These 'control' experiments showed that the cells became injured, in some cases, after soaking for three days in water alone, so it was thought unwise to experiment over periods much above those quoted later. In consequence of this the influence of very dilute solutions of the salts could not be studied.

Rough experiments were first made for each concentration of electrolytic solution used. If, after a certain time of immersion no blue appeared upon the application of the ferric chloride, either the change in the protoplasm had not taken place, or the tannin had had time to diffuse out of the cell. To find which of these possibilities was correct one could either pour a little alcohol over the solution, or heat the slide to kill the cells, to allow the iron compound to pass in, if it had not already been able to do so.

These rough tests served to give the time needed for the change in permeability between limits of half to two hours, according to the nature of the experiment.

The sections in the bottles already mentioned were accordingly left undisturbed for the shorter time limits, and specimens were then removed at intervals of five minutes to twenty minutes according to circumstances;

and tested. Care was taken that all these sections were from leaves of as nearly as possible the same size and condition, in the same rosette of the plant.

All experiments were carried on at the ordinary room temperature, and no corrections were possible for the effect this somewhat fluctuating temperature might have upon the permeability.

#### OBSERVATIONS.

If the rectangular cells were examined from time to time certain changes could be seen. The first thing to be noticed was the fact that the protoplasm, after short immersion in the electrolytic solution, shrank away from the cell wall and formed threads and spherical masses. Treatment with pure water at these early stages was sufficient to restore the protoplasm to its normal form, so that these changes were connected with osmotic changes.

After a certain period of immersion in each case, when the sections were removed and treated with the ferric chloride, distinct blue coloration was seen in the plasmolysed masses of the protoplasm. Cells examined at this stage of immersion without ferric chloride showed a very granular appearance in the protoplasm, this effect being especially marked in the experiments with barium chloride and nitrate.

It was found that periods of immersion which allowed of blue tinting being obtained in the masses of protoplasm also served approximately to render the cell membrane permeable to the tannin within it, because a blue liquid was often seen also in the space between the shrunken protoplasm and the cell wall.

Although permeability to both tannin and ferric chloride seemed to be produced by approximately the same periods of immersion, the membrane did not become permeable, at this stage, to the red material so frequently found in cells of tannin-bearing plants. In this particular plant the red pigment occurs in cells which do not actually contain the tannin, and is particularly abundant in early spring. This colouring matter is soluble in water, and passes out of the cell after longer periods in the electrolytes.

It was frequent to obtain sections showing tracts of cells stained deep blue by the tannin reaction, side by side with tracts showing a clear rose colour. A stronger iron solution than that used for the tests was able to combine with the rose pigment and produce a greenish-brown precipitate within the special cells.

Results given below show details for five electrolytic solutions which were found capable of rendering the protoplasm permeable to the 0·2 per cent. iron chloride. It is intended to carry out tests on a series of salts to find whether there is any general connexion between concentration and time of immersion necessary to produce the given change.

RESULTS.

No. 1.  $\text{Al}_2\text{Cl}_6 \cdot 2\text{H}_2\text{O}$ .

Concentration in grm.-mols.	Date of Experiment.	Critical Time in Hrs.	Average T.	Log T.	Log C + 1.
0.31	Dec. 7, 1916	0.33	0.33	1.518	0.491
	" 8	0.33			
	" 8	0.33			
0.207	Nov. 23	1.16	1.29	0.11	0.316
	" 27	1.25			
	" 27	1.25			
0.155	Dec. 1	1.5	3.25	0.511	0.190
	Nov. 27	3			
	Dec. 1	3.5			
0.103	" 1	11.5	11.37	1.055	0.013
	" 4	11.25			
0.069	" 3	26.5	26.53	1.423	1.838
	" 6	26.6			
	" 11	26.5			
	" 11	49			
0.044	" 14	48.75	48.87	1.688	1.643

No. 2.  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ .

1.227	Nov. 18	0.25	0.23	1.36	1.088
	" 18	0.2			
0.818	" 20	0.25	1.66	0.22	0.92
	Oct. 26	1.66			
	" 27	1.66			
0.545	" 27	2.91	3.05	0.48	0.73
	" 30	3			
	" 22	3.25			
0.409	Nov. 2	7.5	7.19	0.856	0.61
	" 3	7.5			
	" 12	7			
	" 12	6.75			
0.272	" 2	10.5	10.83	1.03	0.43
	" 5	11			
	" 6	11			
	" 10	12			
0.204	" 11	12	11.9	1.075	0.31
	" 12	11.75			
	" 13	23.5			
0.163	" 9	23.5	23.9	1.38	0.21
	" 13	24.3			
	" 15	24			
0.081	" 20	30.5	30.5	1.484	1.908
	" 23	30.5			

No. 3. KCl.

2.01	Feb. 22, 1917	0.25	0.25	1.397	1.313
	" 22	0.25			
	" 22	0.25			
1.34	" 1	1	1	0	1.127
	" 2	1			
0.893	Jan. 15	2.5	2.83	0.452	0.95
	" 18	3			
	" 19	3			
0.67	" 22	4.75	4.86	0.686	0.826
	" 25	4.83			
	" 26	5			
0.447	" 24	8.75			
	" 28	8.66	8.72	0.94	0.65
	" 29	8.75			

Concentration in grm.-mols.	Date of Experiment.	Critical Time in Hrs.	Average T.	Log T.	Log C + 1.
0.337	Jan. 29, 1917	11.75			
	Feb. 1	12	11.83	1.07	0.527
0.268	" 2	11.75			
	" 19	18			
	" 20	18.5	18.25	1.261	0.428
0.201	" 21	18.25			
	" 22	23			
	" 26	22.42	22.75	1.357	0.303
	Mar. 6	22.83			

No. 4.  $\text{KNO}_3$ .

1	May 2	0.33	0.3	1.47	1
	" 3	0.33			
	" 3	0.25			
0.66	" 2	0.91			
	" 3	1	0.97	1.98	0.819
	" 3	1			
0.5	" 7	2.5			
	" 9	2.5	2.58	0.41	0.699
	" 10	2.75			
0.33	" 7	6.5			
	" 9	6.25	6.42	0.807	0.518
	" 10	6.5			
0.25	" 10	10			
	" 18	9.25	9.7	0.98	0.397
	" 21	9.75			
0.2	" 13	24.5			
	" 16	24.25	24.33	1.39	0.301
	" 16	24.25			

No. 5.  $\text{Ba}(\text{NO}_3)_2$ .

0.23	May 24	5			
	" 31	5	5	0.699	0.36
	" 31	5			
0.15	" 31	8			
	June 1	7.75	7.94	0.899	0.17
	" 4	8.25			
	" 6	7.75			
0.11	" 6	12.25			
	" 7	12.5	12.5	1.097	0.04
	" 11	12.75			
0.075	" 10	22.5			
	" 13	22.75	22.62	1.35	1.87
0.058	" 12	24.5			
	" 13	24	24.4	1.387	1.76
	" 13	24.75			
0.046	" 19	33			
	" 24	34.5	33.9	1.53	1.66
	" 26	34.25			

## RESULTS.

Fig. 1 shows the curves obtained for aluminium chloride (I), barium chloride (II), potassium chloride (III), barium nitrate (IV), and potassium nitrate (V).

In each case the concentration  $C$ , expressed in gram-mols per litre, is plotted horizontally, while the time  $T$  needed to produce the change is plotted vertically.

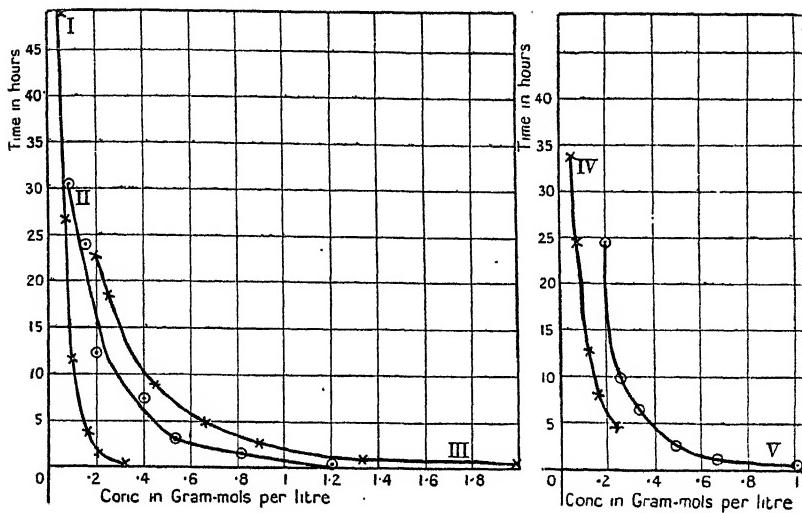


FIG. 1.

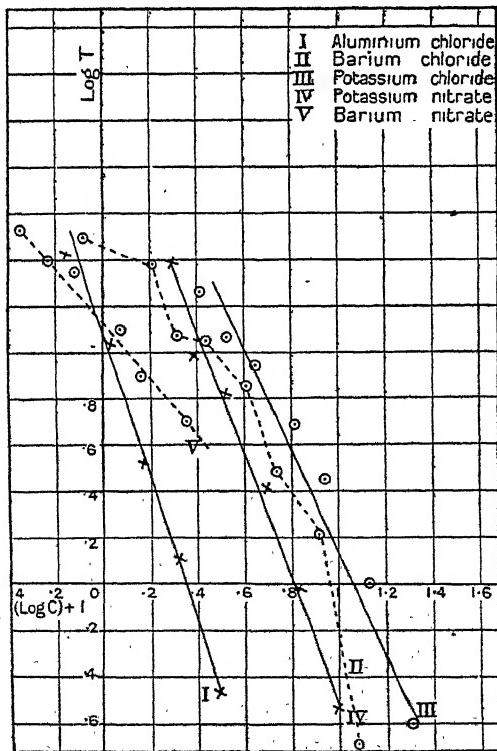


FIG. 2.

The curves obtained suggest the following possible equations:

$$(a) \quad Y = \frac{A}{X^m}$$

$$(b) \quad Y = Ae^{-mx}$$

In the case of (a) the equation can be written:

$$\log Y = \log A - m \log x, \text{ or}$$

$$\log Y = \text{constant} - m \log x.$$

while (b) can be written:

$$\log Y = \log A - mx, \text{ viz.}$$

$$\log Y = \text{constant} - mx.$$

Fig. 2 shows the graphs obtained when logs of the critical times are plotted vertically and values of  $\log (C) + 1$  are plotted horizontally. ( $\log C$ ) + 1 is used instead of  $\log C$  merely for convenience, to avoid so great a number of negative values.

It will be seen that for all the substances examined, except barium chloride, an approximation to a straight line is obtained for these graphs in Fig. 2, suggesting that over the limited periods studied

$$\log T = \text{constant} - A ((\log C) + 1), \text{ where}$$

$T$  = time to produce the change in permeability,

$C$  = concentration in gram-mols per litre.

#### SUMMARY.

1. Immersion in certain solutions of electrolytes was found to produce permeability to 0·2 per cent. ferric chloride in cells of 'London Pride' petioles.

2. The entrance of the ferric chloride was indicated by its reaction with the tannin contained in these cells.

3. The time of immersion needed to produce the abnormal permeability depended upon (a) the concentration employed, (b) the electrolyte chosen.

4. For aluminium chloride, potassium chloride, potassium nitrate, and barium nitrate, results were obtained which suggested approximations to the relationship:

$$\log T = \text{constant} - A ((\log C) + 1)$$

where  $T$  = time of immersion needed to render membrane permeable to 0·2 per cent. ferric chloride,

$C$  = concentration in gram-mols per litre,

$A$  = constant depending upon electrolyte used.

5. Abnormal permeability with regard to the iron chloride could be produced without the membrane becoming permeable to a rose-coloured material frequent in the sap of the cells.

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## NOTE.

ON CELL-REGENERATION IN BOTRYTIS CINEREA.—The formation of cell-membranes as a response to traumatic stimuli has been very completely investigated in many groups of plants,<sup>1</sup> but with the exception of certain Mucorineae<sup>2</sup> little is known of this process in the Fungi. During the course of an investigation of *Botrytis cinerea* it was necessary to examine minutely fragments of mature growths teased out in water. Many of these preparations were maintained for several days, and regenerative changes in the injured mycelium were frequently noted. In these observations, which were merely incidental to the main work, no attempt was made to do more than record the behaviour of the living fungus, occasionally aid being sought in the use of *intra-vitam* stains such as neutral red, methyl violet, and especially Congo red.

The healing process and the rapidity with which it occurs depend to a large extent upon the age and condition of the particular cells affected. Speaking generally, hyphae which have been starved show less regenerative power than mycelium growing upon a physiologically suitable pabulum. No obvious effect of temperature or light was noted, although a more thorough examination devoted to this particular purpose might reveal its presence. Different regions of the same mycelium react unequally to approximately the same type of injury, the regenerative response apparently being related largely to the condition of vacuolation of the protoplast. Young actively-growing vegetative cells are usually filled with non-vacuolate homogeneous protoplasm, whilst of the older cells, those retaining cell-content usually exhibit a multi-vacuolate, honeycomb-like appearance. The conidiophores are highly specialized organs of strictly limited growth. The basal region is tinted and consists of mechanically rigid thick-walled cells in which the protoplast is disposed as a thin parietal layer about one or infrequently two large vacuoles. The upper sporogenous region is hyaline and thin-walled, and the cells are densely filled with homogeneous, finely granular or minutely vacuolate protoplasm in an extremely active condition. The latter cells possess by far the greatest capacity for regeneration: the vegetative hyphae and particularly the older vacuolate cells show this potentiality to a markedly less degree; whilst the cells of the lower region of the conidiophore are almost devoid of it.

In general the types of injury which have been observed to stimulate a healing reaction may be grouped into three categories; (1) acute flexion of cells; (2) punctures and relatively minute wounds; (3) relatively large superficial lesions. In certain injuries two distinct regenerative processes are involved: restitution of the original

<sup>1</sup> See Küster, E.: *Pathologische Pflanzenanatomie*, Jena, 1903, where the important literature is cited.

<sup>2</sup> Cf. especially Van Tieghem, P.: *Ann. Sc. Nat. Bot.*, 1875.

cell-wall and the formation of a completely new membrane about the escaped protoplast.

*Flexion of the hypha.* When a hypha is acutely flexed the bending almost always occurs in the middle region of a cell. If the angle formed be less than forty-five degrees the cell-contents tend to separate into two portions, each of which rounds off its new surface, over which a membrane is formed. The flexion in the hypha tends to become rigid and permanent and the membrane at the point of bending stains more deeply with Congo red.

*Punctures and minute wounds.* If a cell be punctured or minutely wounded a thin stream of cell-content issues from the lesion and diffuses into the surrounding medium. The force of this jet gradually lessens and finally ceases, presumably with the equilibration of endosmotic and exosmotic forces, and the lesion becomes covered by a new membrane, so that frequently the region of injury can no longer be detected. Wounds of a diameter two-thirds that of the filament have been observed to heal in this way, and in some of the wider hyphae the exact manner of membrane restitution may be observed. Usually the new cell-wall substance is laid down simultaneously over the whole surface of exposed protoplasm, but occasionally regeneration proceeds by a centripetal diaphragm-like growth from the edges of the wound.

*Large wounds.* Injuries greater in diameter than from one-half to two-thirds that of the cell rarely heal by restitution of the original membrane, and often the entire cell-content diffuses into the surrounding medium. If, however, the wound be situated towards one end of the cell a certain quantity of protoplasm may remain in the other end, and this either dies *in situ* or rounds off its naked surface and forms a transverse septum closing itself in. More rarely this moiety of cell-content has been observed to undergo a process of rejuvenescence and clothe itself in a completely new cell-membrane, forming what may be regarded as an aplanospore. The latter may germinate, producing a hypha which either pierces the original cell-wall, simulating a normal hyphal branch, or grows longitudinally through the parental filament, living apparently in a parasitic manner.

Not infrequently a hypha is sheared across so that a cell is divided cleanly into two portions. The protoplasm usually diffuses from the open ends, but one or both moieties have been observed to round off their naked surfaces and form closing septa.

Severe crushing or considerable laceration of a cell usually results in the death of the protoplast.

*Membrane formation by free protoplast.* As a rule when a cell is injured part or all of the cell-content is ejected and is lost by diffusion into the surrounding medium. Rarely, however, the entire protoplast is released as a single unit, and of three such cases which were noted, one died, and two assumed a spherical shape and formed about themselves new cell-walls. Subsequently they elongated into slender hyphae giving rise to growths indistinguishable from rather delicate normal mycelium.

In all instances of cell-restitution such as have been described growth proceeds only after the enclosure of the exposed protoplasmic surface by a cell-membrane. In the case about to be described, however, this procedure was reversed and a certain amount of free protoplasmic growth occurred prior to cell-wall formation.

A water preparation was made about noon on Wednesday, retained in a moist condition and re-examined four and a half hours later. It was then noted that a moiety of protoplasm remaining in the lower end of a cell situated at the sporogenous tip of a mature conidiophore had not rounded itself off in the usual manner, but showed marked pseudopodial elongations of its naked surface, one such lobe having penetrated the membrane of the parental cell (Fig. 1).<sup>1</sup> The adjacent cell in the conidiophore was minutely vacuolate and perfectly normal in appearance. The lower portion of the free protoplasm in the injured cell contained a number of minute granules and a few small vacuoles. At the naked growing surface the protoplasm was homogeneous. The lobular prolongations were clear and definite, and, with the exception that no trace of a streaming movement could be detected, bore a striking resemblance to those of an Amoeba. The structure was unmistakably a pseudopodial growth of a naked surface of protoplasm. By noon the following day the

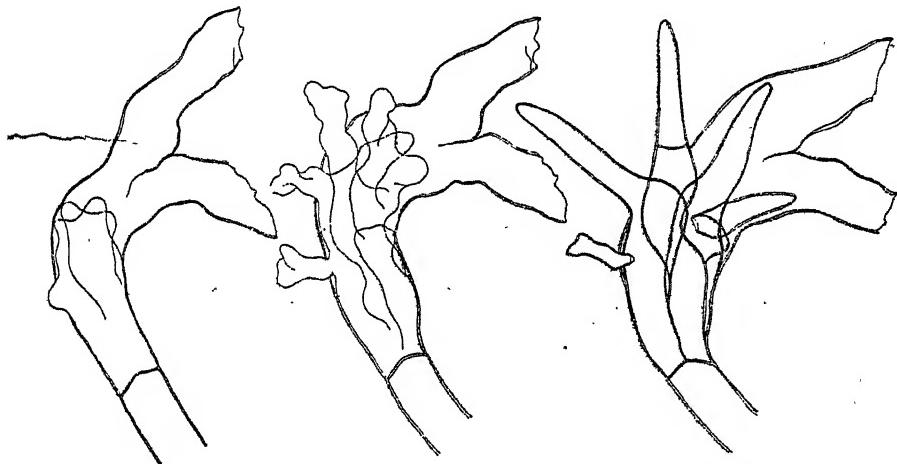


FIG. 1.

FIG. 2.

FIG. 3.

protoplasm had increased to about twice its original bulk, and the irregularly-lobed pseudopodia were very marked, several having pierced the parental sheath (Fig. 2). Many more minute vacuoles were present in the lower region, whilst along the middle of the lobes and scattered generally through the substance of the protoplasm were large numbers of very fine granules. The edges of the lobes were hyaline and homogeneous. The preparation was again examined at 2.20 p.m. on Friday, when the facies of the specimen was found to have completely changed (Fig. 3). The irregularly-lobed naked pseudopodia had become smoothly digitiform, and were enclosed in thin but quite definite cell-membranes which extended down to the wall of the adjoining cell. In one of the processes a fine transverse septum was visible, and the entire structure merely formed a cluster of minute and delicate radiating hyphae arising in an injured conidiophore. By 10 a.m. the following day the three

<sup>1</sup> The drawings were made with a Zeiss camera lucida; and Swift one-eighth objective NAO. 92 with No. 8 compensating oculi were used. They have been reduced to one-half.

most prominent hyphae had developed into slender filaments bearing lateral branches; and subsequently all the processes gave rise to normal mycelium.

If water preparations of *Botrytis* conidiophores be maintained for some days, clustered hyphal growths originating in wounded sporogenous tips and identical with that noted above are not rare. The development of such structures by the more usual regenerative processes has not been observed, and it does not appear improbable that they may arise in the same manner as the one described.

The protoplasm of the sporogenous tips of the conidiophores exists in an extremely active and plastic condition, this being exemplified by its immense regenerative capacity; and, as is evident in the present specimen, this protoplasm is able, under certain conditions and for a period of time, to live and grow when part of its surface is in a free plasmodial state, and only subsequently enclosed in a cell-wall.

If this potentiality be not restricted to the fruiting hyphae of *Botrytis cinerea*, but be distributed widely among the Fungi, and in particular be an attribute of the vegetative mycelium of pathogenic forms, it will have an important bearing upon general ideas of fungal morphology.

That there is no inherent impossibility in the growth and development of naked protoplasm is evident in the existence of the Protozoa and Myxomycetes. Even in the Fungi proper there are forms, such as many Synchytriaceae, in which the organisms live for the greater part of their life as protoplasmic masses free from any enclosing cell-membrane. In the Bacteria, organisms probably of a degenerate fungal nature, evidence has recently been presented<sup>1</sup> of a free plasmodial or 'symplastic' state.

In a research on the behaviour and morphology of the vegetative mycelium of *Botrytis cinerea* in the tissues of *Aesculus Pavia*, which is now being prepared for publication, it was found that certain of the hyphae were in a naked condition, existing as free protoplasmic substance.

Finally it may be noted that for years the Swedish school of phytopathologists have persistently maintained the conception of mycoplasm, and quite recently Eriksson has used his hypothesis in the elucidation of the many problems of the causation of the potato blight by *Phytophthora infestans*.<sup>2</sup> Mycoplasm involves far more than the mere existence of fungus protoplasm in a naked state, and the merging and subsequent dissociation of the individualities of host and fungus, which is the vital part of this hypothesis, must still be regarded as 'non-proven'. It would seem, however, somewhat injudicious merely to ignore every aspect of this idea or summarily dismiss it as impossible, for in the light of what has been written above it may be that buried beneath much interpretation there is a basis of fact.

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